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# TABLE OF CONTENTS

## No 1 January 1943

Cortical response of the anesthetized cat to gross photic and electrical afferent stimulation W H MARSHALL, S A TALBOT AND H W ADES	1
The effect of skeletal fixation on skeletal muscle D Y SOLANDT, RUTH C PARTRIDGE AND JOHN HUNTER	17
Intravenous potassium, calcium and magnesium and the cortical electrogram of the cat M A RUBIN, H E HOFF, A W WINKLER AND P K SMITH	23
Thermoregulatory pathways in the cat brain stem L E BEATON, C R LEININGER AND W A MCKINLEY	29
Spinal distribution of thermoregulatory pathways in the monkey L E BEATON AND C R LEININGER	37
The response of single auditory-nerve fibers to acoustic stimulation R GALAMBOS AND H DAVIS	39
A secondary acoustic area in the cerebral cortex of the cat H W ADES	59
The pseudoaffective state and decerebrate rigidity in the sloth S W BRITTON AND R F KLINE	65

## No 2 March 1943

Investigation of epileptiform attacks produced by sudden cooling of frog spinal cord M OZORIO DE ALMEIDA	73
Effect of hypothalamic lesions on electrical activity of cerebral cortex S OBRADOR	81
Electrical polarization of pacemaker neurons T H BULLOCK, H S BURR AND L F NIMS	85
Specific excitability of the endplate region in normal and denervated muscle S W KUFFLER	99
Reflex action in relation to pattern and peripheral source of afferent stimulation D P C LLOYD	111
Functional organization of temporal lobe of monkey ( <i>Macaca mulatta</i> ) and chimpanzee ( <i>Pan satyrus</i> ) P BAILEY, G VON BONIN, H W GAROL AND W S McCULLOCH	121
Long association fibers in cerebral hemispheres of monkey and chimpanzee P BAILEY, G VON BONIN, H W GAROL AND W S McCULLOCH	129
The effect of oxygen tension on the metabolism of cerebral cortex, medulla and spinal cord F N CRAIG AND H K BEECHER	135
The interconnection of antidromic and orthodromic volleys in a segmental spinal motor nucleus D P C LLOYD	143

## No 3 May 1943

The monkey ( <i>Macaca mulatta</i> ) after hemisection and subsequent transection of spinal cord G P McCOUCH, J HUGHES AND WINIFRED B STEWART	155
The effect of insulin hypoglycemia on conditioned reflexes E GELLHORN AND H MINATOYA	161
Cortical localization of symbolic processes in the rat II Effect of cortical lesions upon delayed alternation in the rat C T MORGAN AND W M WOOD	173
Motor response to stimulation of cerebral cortex in absence of areas 4 and 6 ( <i>Macaca mulatta</i> ) M A KENNARD AND W S McCULLOCH	181
Sweat gland responses to sympathetic stimulation studied by the galvanic skin reflex method C P RICHTER AND F WHELAN	191
Localization of the salivatory center in the medulla of the cat S W WANG	195
Localization of enzymes in nerves II Respiratory enzymes D NACHMANSON, H B STEINBACH, A L MACHADO AND S SPIEGELMAN	203
Mechanism of temporal fusion Effect of photic stimulation on electrical activity of visual structures A E WALKER, J I WOOLF, W C HALSTEAD AND T J CASE	213

## No 4 July 1943

Stephen Walter Ranson An appreciation	223
Temperature effects on reflexes of isolated spinal cord Heat paralysis and cold paralysis M OZORIO DE ALMEIDA	225
Electroencephalogram of decorticate monkeys M A KENNARD	233
Spontaneous electrical activity of thalamus and other forebrain structures R S MORISON, K H FINLEY AND G N LOTHROP	243
Acute physiological effects of gunshot and other penetrating wounds of the brain J E WEBSTER AND E S GURDJIAN	255
Experimental investigation of visceral afferent synapses in coeliac ganglia S FREUND AND D SHEEHAN	263
Pressure block in nerves provided with arterial sleeves P WEISS AND H DAVIS	269
Note on organization of the tactile sensory area of cerebral cortex of chimpanzee C N WOOLSEY, W H MARSHALL AND P BARD	287
Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat D P C LLOYD	293
Conduction and synaptic transmission of reflex response to stretch in spinal cats D P C LLOYD	317

## No 5-6 September-November 1943

Acetylcholine level of rat cerebral cortex under conditions of anoxia and hypoglycemia J H WELSH	329
Accommodation and autorhythmic mechanism in single sensory fibres R GRANIT AND C R SKOGLUND	337

The relation of area 13 on orbital surface of frontal lobes to hyperactivity and hyperphagia in monkeys T C RUCH AND H A SHENKIN	349
Responses to electrical stimulation of single sensory units of skin G H BISHOP	361
Action potential and enzyme activity in the electric organ of <i>Electrophorus electricus</i> II Phosphocreatine as energy source of the action potential D NACHMANSOHN, R T COX, C W COATES AND A L MACHADO	383
The formation of acetylcholine A new enzyme "Choline acetylase" D NACHMAN- SOHN AND A L MACHADO	397
Effects of EEG of chronic lesions of basal ganglia, thalamus and hypothalamus of monkeys M A KENNARD	405
Hand and foot patterns of low electrical skin resistance Their anatomical and neu- rological significance C P RICHTER, B G WOODRUFF AND B E EATON	417
Nature of paresis following lateral cortico-spinal section in monkeys B W CANNON, L F BEATON AND S W RANSON, JR.	425
Removal of acetylcholine by cholinesterase injections and the effect thereof on nerve impulse transmission B MENDEL AND R D HAWKINS	431
The basis for repetitive activity in phrenic motoneurons R F PITTS	439
Index	455



# CORTICAL RESPONSE OF THE ANESTHETIZED CAT TO GROSS PHOTIC AND ELECTRICAL AFFERENT STIMULATION\*

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## INTRODUCTION

THESE EXPERIMENTS were undertaken to determine some of the general aspects of the cortical reactions to photic and electrical stimulation of the visual system in the cat. Bartley and Bishop (2) first observed that the response of the rabbit's cortex to electrical stimulation of the optic nerve consisted of a complex series of events. The cat's cortex, which is somewhat more interesting for purposes of analysis, has been explored in different ways by several experimenters. Kornmüller (12) observed that the spontaneous rhythms could be modified by photic stimulation, and that "on" and "off" responses could be superimposed on the rhythms. The extent of the cortical area which gave a more or less characteristic rhythm and definite responses was mapped. Gerard, Marshall and Saul (10, 11) made somewhat similar observations and explored the extent of the area giving visual response. They used concentric needle electrodes and a loud speaker as indicating device. They reported a larger area than that found by Kornmüller. Their area more nearly resembled that suggested by Poliak (20), including what he then labeled as striate and extra-striate visual cortex. Also in disagreement with Kornmüller's restriction of visual responses to area 17, Talbot (21, 23, 24) found "primary" visual responses in the cat to be evoked locally in both 17 and 18 by photic stimuli localized in the field. O'Leary and Bishop (18, 19) mapped the apparent visual projection area in the rabbit using electric shocks to stimulate the optic nerve. They found that both striate and an area usually considered to be parastriate were involved. Claes (7) reported observations on the visual system of the cat made on the isolated encephalon preparation.

The present experiments were essentially a continuation of the observations on the primary projection system of the somatic sensory system by Marshall, Woolsey, and Bard (13, 14, 26).

## METHOD

Action potentials were led from the pial surface by cotton thread electrodes which were connected to a two channel cathode-ray recording system. The electrodes used were "monopolar," though many checks were done with "bipolar" leads subtending a short distance on the surface of the cortex, or vertically oriented on and within the cortex. The electrodes were held in a modified Horsley-Clarke stereotaxic instrument. Various kinds of photic stimulation were used. For many experiments a neon lamp driven by a pulse

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generator was employed. For others an incandescent lamp was focused on a translucent light scattering cover placed as a contact lens on the cornea, and the beam was interrupted by a rotating slit. The duration of flash was from 1 msec upward. The inside of the cover had a brightness of 30 to 250 mL and was equivalent to illuminating the entire visual field at these levels. Usually only monocular stimulation was used, but in some experiments both eyes were stimulated simultaneously with light of about the same intensity by splitting the beam from the incandescent lamp. Observations were also made on the cortical potentials evoked by stimulation of the optic nerve by single electrical shocks. Nembutal was usually employed as a general anesthetic, but in some experiments ether was used. Observations on the amplitude of the response, sensitivity, threshold, and latency were made at various depths of anesthesia. In the earlier experiments of this series the pupil was dilated by atropine after it was observed that atropine had an exciting effect on the cortex (22) it was discontinued and hyoscine was used instead.

Intracortical and subcortical recording was accomplished by small steel suture needles insulated to the tip, or by electrodes consisting of No. 40 enameled stainless steel wire with an outside diameter of 75 micra. Since the Horsley-Clarke apparatus was constructed of stainless steel this electrode arrangement was relatively free from polarizing voltages. Points at which observations were taken were marked by a modification of the iron deposition method of Hess (15). For some of the observations the optic nerve was stimulated by single shocks. For this purpose the eye was resected, the retina cut off around the optic disc and the remnants of the retina were then tied to a silver wire. Another silver wire supported on a universal joint constituted the second stimulating electrode.

## RESULTS

The sign of the electrical response at the cat's cortex following photic stimulation is most typically, but not always, initially surface positive, and has a latency of 17 to 25 msec. Any stimulation of moderate to high intensity produced dorsally more than one surface positive wave, the interval between them being 40 to 80 msec. As many as 5 such responses often occurred, following a single brief photic stimulation. It is doubtful that a 2 msec flash produces an "off" effect, but the multiple response occurs nevertheless (4, 22). Multiple responses are obtained even with long flashes 200 to 300 msec in duration. While the analysis of the multiple response phenomenon is not the concern of this paper, it should be pointed out that it is readily elicited under anesthesia of such depth that spontaneous activity is negligible.

With nembutal anesthesia, there appear to be two general types of cat preparations based on characteristics of the cortical response. The first type is the most numerous and gives responses which are predominantly surface positive with a comparatively small negative component following each positive wave. The second type of preparation yields potentials in which the predominant phase is negative. Apparently either the negative wave may begin a few msec after the initiation of the positive wave, and thus cancels a considerable part of the positive component of the response, or the positive wave may be intrinsically smaller. Occasionally the initial positive phase is insignificant. It occurred to us that this difference in characteristics might be correlated with the fact that some cats show an initial excitement stage while going into nembutal narcosis. In some of the animals this reaction is comparable to the reaction following morphine administration in the cat. We have, therefore, made it a point to compare the type of response to photic

stimulation in all cats which show this reaction, but there is no correlation. An example of the predominantly negative type of response is shown in Fig 1-2 and 8, in which it is seen that the first response of the series is definitely diphasic with positive phase first. The second response is initially negative with an ensuing positive phase at certain points on the dorsal striate area. At other points it is negative with no appreciable positive phase. In some preparations all positive phases are small or negligible.

In general, however, in moderately or deeply nembutalized preparations the initial response is a definite positive wave. Under light nembutal anesthesia the succeeding negative wave is usually more prominent, if more anesthetic is administered (intraperitoneally or intravenously) the negative wave is usually reduced or obliterated, leaving the typical predominantly positive wave characteristic of most of the primary sensory reactions of the cortex in somatic, auditory or visual systems. Apparently, the negative wave of the response tends to be reduced by the barbiturates along with the spontaneous cortical potentials. The clearest demonstration of the difference between the positive and negative components in this sensory reaction is that shown by the picrotoxin-cocaine reaction of Curtis (8). The picrotoxin is applied to a spot on the striate cortex and after several minutes the initial positive wave is enhanced and a large negative wave develops. If a depressing agent such as nembutal is now applied on the same spot, the negative wave quickly disappears leaving the enhanced positive wave.

It is not certain that there is discharge over projections from the striate cortex to either the tectum or the suprasylvian gyrus in typical deeply anesthetized preparations in which the amplitude of the negative phase of the response is negligible. If picrotoxin is applied, however, the negative wave produced by the drug in the striate area is accompanied by obvious electrical responses or definite changes of activity in the tectal and pretectal region and on the suprasylvian gyrus. If nembutal is now applied to the same spot the negative wave disappears and the response observed in the tectum or the suprasylvian gyrus always becomes small and usually disappears. This relation is not always diagrammatically clear however, and some observations indicate that the negative wave apparently was eliminated in the striate area without completely eliminating the associated response which was initiated or facilitated by the picrotoxin. This may only mean that some negativity which still remains is cancelled out by the enhanced positive wave and so does not appear on the records. We have not thoroughly explored the thalamus and lateral geniculate for evidence that the cortical projection reaction initiated by picrotoxin is actually projected to those regions. Certainly, in the lateral geniculate it is not obvious, and this fact agrees with the lack of anatomical evidence that the striate projects to the geniculate of the cat.

The hypothesis that different mechanisms are responsible for the positive and negative waves is given support by observations on the interaction between two eyes. This has been done by electrically stimulating the



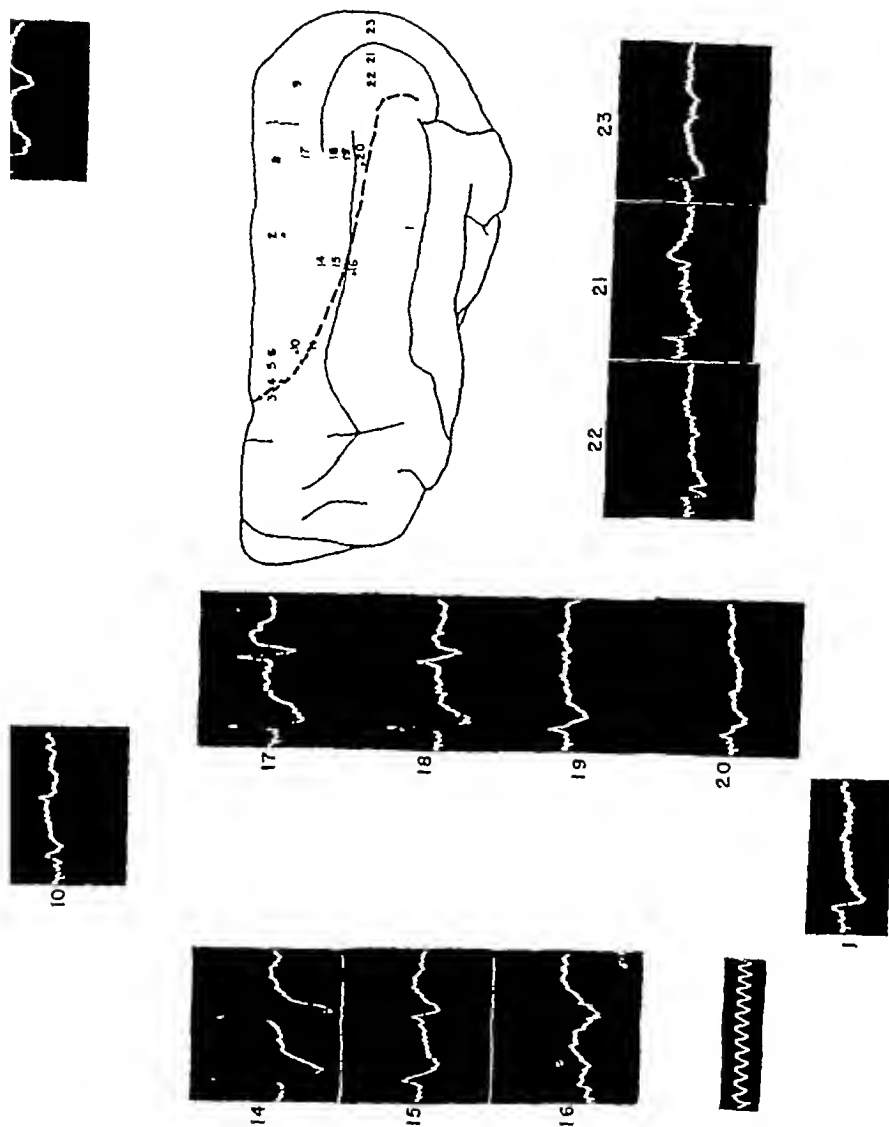


Fig 1 Typical distribution of cat's cortical response to strong photic stimulus 10 msec in duration Shortest latency from start of stimulus to initiation of atrate response about 17 msec Note in record from point 17 that second response is initially negative Second response from points 8 and 9 entirely negative

optic nerve on one side at various intervals before and after photic stimulation is applied to the other eye. If the photic response is approximately completed when the electrical shock is delivered at the opposite nerve, the surface positive cortical response to the latter is considerably subnormal, but

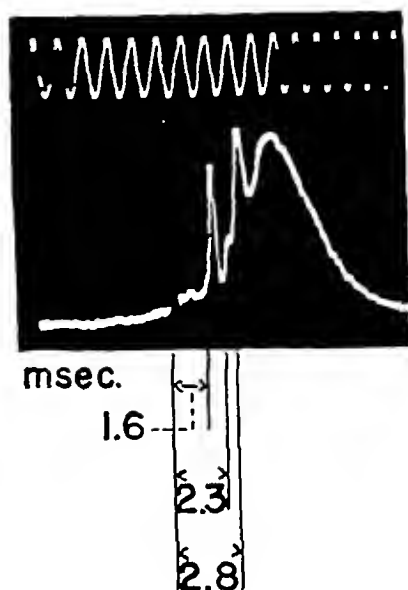
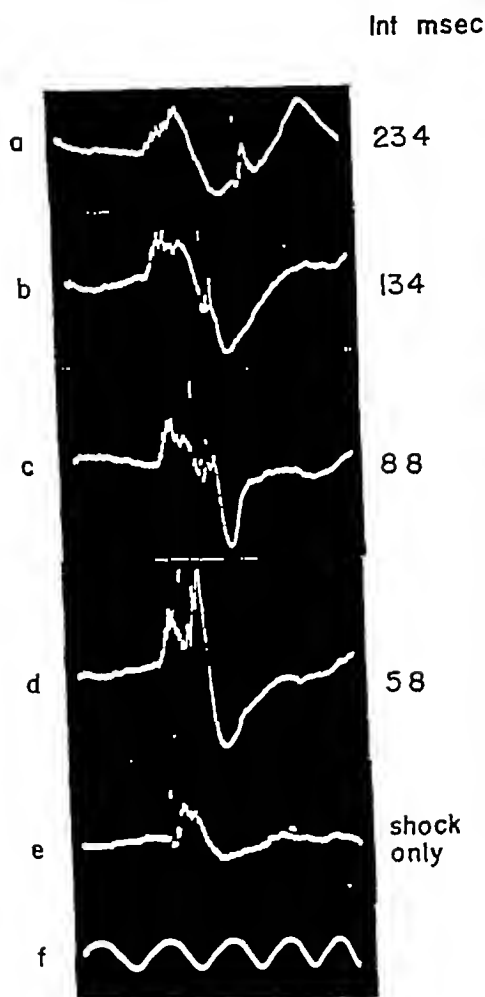


FIG 3 Striate response to single shock applied to contralateral optic nerve. See text.

FIG 2 Showing substantial degree of independence of the positive and negative response mechanisms. Photic response leading a response to single weak electric shock on the contralateral optic nerve. The intervals between the initiation of the photic response and the application of the shock are indicated at right of oscillogram. At *a* the positive phase of electric shock is subnormal and negative phase absent. *b* shows 4th component absent but negative phase enhanced. *c* more enhancement of last positive and negative phase. *e* is response to the electric shock only.

the negative wave following it may show significant enhancement both in amplitude and gradients of the waves (Fig 2).

The composition of the cortical wave obtained by photic stimulation is probably the result of algebraic addition and temporal dispersion of the spike wave series evoked by electrical stimulation of the optic nerve, Fig 3.

The latter reaction consists of three spikes of axon time-dimensions, and a slower wave. The first spike starts with a latency of about 1.5 msec. The second is typically a smaller one occurring about 0.8 msec. after the initiation of the first spike, and on or near its peak the third spike begins. The third spike, perhaps also the second spike, is superimposed on the rising phase of the slow wave, and its peak occurs about 1.2 msec. after the beginning of the first spike. The slow surface-positive wave appears to start rising from the base line about the time at which the first spike process is concluded. It is usually followed by a more or less prominent negative wave, and, as in the case of the photic response, the negative wave is sometimes more prominent than the positive wave.

This spike wave is similar to that described by Bishop and O'Leary (5). There may, however, be a disagreement regarding what we designate as the second spike. In our records it almost always occurs as shown in Fig. 3. Occasionally on contralateral stimulation, but more often when the ipsilateral nerve is stimulated, and perhaps dependent on the position of the lead on the visual cortex, the second spike is temporally separated from the third spike so that it is clearly revealed as a distinct entity. The first and second spikes are both resistant to cortical depression produced by failure of circulation, application to the pia of hypertonic salt solutions, distilled water, or depressing drugs. Both exhibit short recovery times, and when the shock frequency is increased to 20 per sec. both of these spikes are attenuated much less than the succeeding cortical components. These characteristics suggest that each is a geniculate projection reaction. The first spike may represent the invasion of the cortex by the radiation reaction and the second may represent a similar process of a reaction which has been delayed in the lateral geniculate body by transference across interneuronal cells in that nucleus. However, electric shocks applied in the region of the optic radiations near the lateral geniculate produce the same spike pattern as is obtained by stimulation of the optic nerve. While this might result from antidromic effects, the probability is that the second spike is cortical in origin.

If a monopolar steel needle electrode insulated to the tip, or an enameled steel wire 75  $\mu$  in diameter, is slowly pushed into the cortex, fairly definite discontinuities appear in the record at characteristic levels which can be identified by a suitable marking technic (15). On the surface of the pia the spike wave series is seen (Fig. 3). In the upper part of layer III or in layer II the slow surface positive wave is not recorded, in lower III or upper part of layer IV the surface positive wave is recorded as a smaller negative wave. The third spike is not recorded in lower III, and in the lower part of IV it is inverted. Within layer IV the second spike disappears and the first cortical spike shows reduced amplitude. Within the limitations of this evidence, these observations indicate that the fourth (slow) cortical component of the surface response is elaborated mainly in layers II and I, the third component in layers III and II, the second in layer IV and perhaps part of the first component also in layer IV.

The slow surface positive wave is composed of many smaller reactions algebraically added, each of which has a longer duration than the spikes which precede it. This can be seen when the frequency of the stimulus is increased to about 20 per sec., the first and second spike components undergo relatively little reduction, the third spike is seriously reduced, and the fourth component is very small and composed of fluctuating small waves. These wavelets sometimes fluctuate quite regularly, with a long period of about 7 sec., and may be correlated with similar fluctuations in components at the geniculate. When the stimulus is repeated at cycles of 1 per sec. or less, the fourth component often shows 2 or 3 definite inflections, suggesting that it is composed of reactions involving at least three systems of cortical neurons.

#### *General distribution of responses*

There is a striking difference between the amplitude of the response on the posterior half of the gyrus lateralis and the entire gyrus lateralis posterior. On the gyrus lateralis the positive wave is typically much larger than that on the gyrus lateralis posterior and the negative wave following the positive wave is relatively smaller. Observations on sections of brains of some of these cats indicate a significant correlation between the population density of larger cells in layers II and III and the amplitude of the surface positive wave.

The anterior boundary of the gyrus lateralis has been studied with some care and it has been found that electrical responses to photic stimulation occur within 2 or 3 mm. of the junctions of the ansate and marginal sulci, Fig. 1. The apparent visual projection area tends to follow the splenial sulcus as it curves upward at its anterior end. These observations have been checked with differential, or bipolar leads, one electrode being on the surface near the active area and the other just on the edge. The position of the anterior boundary is not a function of depth of anesthesia, whether nembutal or ether is employed. The identification of small responses is more difficult when spontaneous activity is intense, but average observations show that the fundamental reaction of the primary projection system is about the same, though negative components are larger and more definite in light anesthesia. The boundaries correspond closely. We have checked this cortical region anatomically by making small lesions in the ipsilateral cortex and observing retrograde degeneration in the geniculate 10 to 30 days later (Fig. 4). This evidence, while not invulnerable, agrees very well with the electrical evidence and indicates that the geniculate does project somewhat more anteriorly on the dorsal cortex than the maps of Campbell (6), Minkowski (17), and Kornmüller (12) indicate.

In nearly every experiment definite responses were found on the posterior half of the suprasylvian gyrus. Usually they are smaller in amplitude and often their latencies are the same as those of the striate area responses. These responses are seen not only when monopolar leads are used, as some experimenters have reported, but have been regularly observed with bipolar

electrodes oriented in several different ways. They are usually single in moderately deep nembutal anesthesia. The first type of response in the suprasylvian shows many of the characteristics of primary sensory reactions and agrees with the observations made with localized photic stimuli (24). Reactions of this type are particularly common over the entire posterior half of the suprasylvian gyrus. It has not yet proved possible to observe clear degeneration in the lateral geniculate following circumscribed lesions in the suprasylvian gyrus. If there is any such degeneration it is not obvious to us, and is certainly not comparable to the type seen following lesions in the striate area. This matter is still under investigation. However, when enough

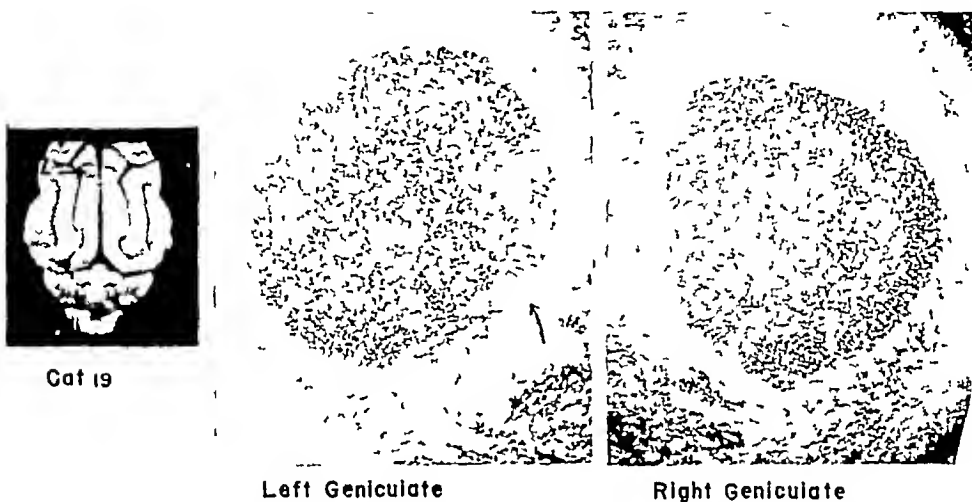


FIG 4 Localized retrograde degeneration reaction in geniculate following lesion in anterior part of marginal gyrus as indicated in picture of brain in upper left. Arrow indicates degenerated region in left geniculate.

time has elapsed following area striata removal for retrograde degeneration to be well developed in the lateral geniculate, responses to photic stimulation are no longer obtained from the suprasylvian.

At various regions of the suprasylvian gyrus, there is often present a second type of response which has considerable amplitude and a longer latency than the primary response. It typically disappears in deep anesthesia. This component is apparently due to activation of association fibers projected from the striate area to the suprasylvian cortex and can be clearly demonstrated by the picrotoxin-nembutal reaction as discussed above.

An area made up of the lateral middle part of the suprasylvian gyrus and often extending over onto the ectosylvian gyrus across the ectosylvian sulcus, shows a curious reaction. It is quite well localized in this region (region 1 on the chart, Fig 1), whether electrical (Fig 5), or photic stimulation is used (Fig 1), and it is usually found ipsilaterally, as well as contralaterally to the

stimulated side Its relation to the visual pattern has not been investigated It persists after both striate cortices have been removed and the tectal region separated from the geniculo-thalamic region by a sagittal knife cut The threshold to electrical stimulation is higher than that for the striate reaction, but is of the same order of magnitude This reaction lacks the spike sequence of the striate reaction (Fig 5)

### DISCUSSION

As many investigators have recognized, the phases of cerebral action potentials can be, at least, partly accounted for on the assumption that impulses approaching the electrode produce positive signs, those leaving the electrode produce negative signs, and that those approaching and passing the electrode show two prominent phases, plus another less prominent one, with positive phase usually first These considerations become more complicated when the neurones (and hence the resulting dipoles) are short compared with the velocity and wavelength of the reaction, or in cases where strata of vertically oriented neurones may be involved as in the cortex The proximity of a non-conducting boundary also enters into the situation In all cases it is probable that the reactions sum to produce a higher potential when the electrode is just over the dipole stratum than when it is within it

It appears to us that these general considerations are just as applicable and, perhaps, more useful for certain problems than attempting to apply rigorously the theoretical treatment of Wilson, McLeod and Barker (25) The smallest electrode we used was  $75\mu$  which is sufficiently large to make killed-end effects significant, and these have the property of exaggerating the first positive phase of approaching reactions, relative to the negative phase of receding reactions How significant this factor is we do not know, but we do not believe it is serious It is worth pointing out that an electrode on the pial surface is virtually a dead end lead, and that one placed in a nucleus is somewhat similar in principle because of the synapse delay factor which temporarily separates the approaching and receding reactions

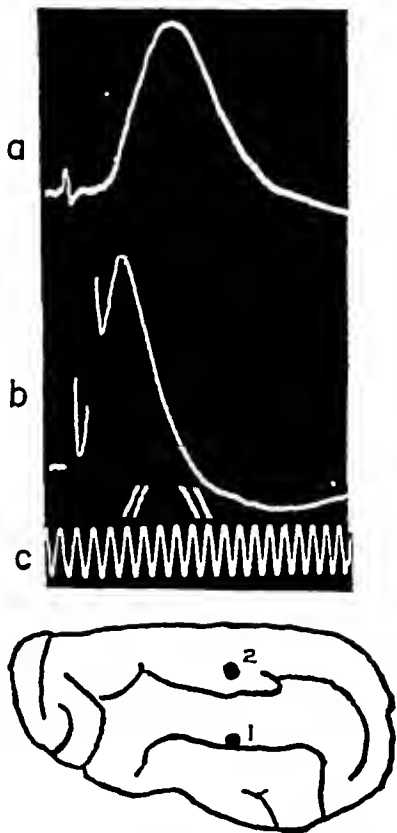


FIG 5 Comparison of response from region 1 (a) and striate response (b) of the suprasylvian to electric shock to contralateral optic nerve C is 1000 cycles

If the dipoles produced by these respective reactions are of a sufficient magnitude, as is the case when recording from the lateral geniculate, the optic tract reaction there and the succeeding radiation reaction may each be the theoretical triphasic spike series with the one major phase of each reaction reversed (see also 16)

Pending further information we suggest that the records indicate that the fourth component can be attributed to activity in the plexiform layer I, the third component to layers II and III and the second component to layer IV. The first component may be considered to be elaborated by radiation endings. The probability is that the second component is the result of synaptic activation of a short dipole confined to layer IV. Negative waves, recorded on the pia, are recorded in reverse phase in the lower layers of the cortex. This agrees with the idea that the ascending electrical processes are surface positive and the descending processes are surface negative.

Exactly what enters into the synthesis of the fourth or wave-like component we cannot say. It is composed of at least three components, and perhaps one of these may arise from the long cell processes which enter the plexiform layer. The tangential component of the reaction in the plexiform layer should be significant for reactions involving this layer. This wave is summed of activities of neurones having longer time parameters than those of the spike-producing neurones. It should be also recognized that, perhaps, in these systems the factor of synapse-time may be radically different than that concerned in the spike reactions. The latter may all have a finite synapse-time similar to that of the geniculate, or the IIIId nerve nucleus. Synapses of the free axon ending types, on the other hand, may have a variable transmission time which under certain conditions of excitation may be zero. This variability would permit temporal dispersion to play a greater role in the elaboration of wave-like responses and hence collaborate in the summation of reactions of small potential into smooth wave shapes.

The fact that photic stimulation sometimes evokes responses which are entirely or predominantly surface negative indicates that under certain conditions, which have not yet been defined, the negative or corticofugal reaction overshadows the positive component, or that the positive components are lacking. Electrical shock stimulus applied to the optic nerve, however, shows that the first three (spike) components are always present and positive. This is probably true also for photic stimulation, but the temporal dispersion, in this case, conceals the detail. The fourth or wave-like component, however, sometimes exhibits only a small positive component which may be followed by a large negative wave, or the negative wave may obviously begin during the rising phase of the positive component and thus partially cancel it. The negative wave, when at its maximum, has a much higher electrical amplitude as indicated by the strychnine and picrotoxin reactions. Hence, it is clear that under favorable conditions for excitation of the corticofugal neurones or descending intra-cortical pathways, and with the temporal dispersion associated with photic responses, that the negative

component of the reaction may completely mask the positive components. While in general, we believe it is correct to speak of the negative reactions as indicating corticofugal activity, it is obvious that descending intracortical reactions would be accompanied by surface negative signs whether the projection neurones are activated or not. There is also the possibility, mentioned by Adrian, that activity propagated tangentially in the cortex may exhibit negative waves. The promptness with which nembutal abolishes the negative picrotoxin reaction suggests, in this case, that the reaction is coupled through the plexiform layer. It is possible, however, that other negative reactions are initiated without the participation of the plexiform layer. This would help to explain why the positive phase is sometimes missing.

Another factor of equal importance in the relative size of the negative phase, is the summation interval of the neurones participating in the negative reactions. The interaction illustrated in Fig. 2 shows this over a fairly short interval. Much longer intervals can regularly be demonstrated by the picrotoxin reaction, if a multiple response to photic stimulation is occurring. When three or more successive surface positive responses are following each flash of light, and when picrotoxin has been applied to the surface of the cortex, the characteristic picrotoxin (or strychnine) effect is seen first on the last response. A few seconds later it moves to the next response, and so by steps, it advances to the first response. This indicates that the positive reactions exert a cumulative effect on the excitability of the neurones involved in the negative response, and that such summation may extend for at least 300 msec. It follows that after several conditioning volleys of thalamic or other origin, that the corticofugal mechanisms may be activated by a comparatively small stimulus. Hence, the positive component may be so small that it is not seen. This may be significant in instances in which "spontaneous" waves of the cortex are, apparently, entirely negative. It also explains why a more prominent negative phase is often seen in the second or third response when the responses are multiple and, also, why the "off" response is often predominantly negative as in Fig. 1, 2 and 8.

Our employment of picrotoxin to produce corticofugal discharges is, in principle, merely a modification of the technic so brilliantly employed by J. G. Dusser de Barenne and his collaborators. Our method of using these drugs is more similar to that of Bartley, O'Leary and Bishop (3), and our application of the method originated with Curtis (9) who found that application of strychnine to the surface of the pia usually enhances any reaction already present and often produces large negative components where none existed previous to the application. The important fact was that under conditions of repetitive stimulus at, say, 1 per sec., the strychnine response tied in with the response to a peripheral tactile stimulus. An area of the somatic sensory cortex, for instance, which was the locus of a surface positive response following a peripheral tactile stimulus, will, after application of the strychnine, yield an enhanced surface positive wave immediately followed by a large negative wave which in turn is accompanied by responses in other



loci of the cerebrum to which association fibers are projected from the strychninized area. This phenomenon differs from that of the Dusser de Barenne school in that, if the stimulus is repeated at a set frequency (often as low as one in 10 sec) few or no uncontrollable spontaneous strychnine discharges occur in preparations anesthetized with usual dosages of nembutal or dial. That is, the system is now equilibrated and operating in a quasi-stationary state. Each stimulus cycle causes the entire response cycle to repeat the pattern recurring with nearly or quite the same characteristics on the successive cathode ray traces. Curtis (8) also applied cocaine to the same spot and thereby temporarily abolished the negative phase of the convulsant drug reaction. This makes it possible conveniently to turn the corticofugal mechanisms on and off. The advantages of this technic for certain types of experiment are obvious.

Curtis tested several excitants (9) and found that picrotoxin is slow to act, but is the most potent, and that its effect persists for several hours, metrazol acts rapidly, but the effect persists for only a few minutes, and strychnine acts in 5-15 min and persists for 30-40 min. Picrotoxin in conjunction with nembutal has been regularly used for these experiments. It is worth pointing out that this technic reduces the necessity of using several parallel recorders with continuous film and triangulation to locate the areas or regions actually reacting. This follows because the reaction is recurring on the oscillograph tubes in approximately the same pattern for each successive stimulus and the "spontaneous strays" which sometimes occur can be immediately identified. In the meantime, electrodes can be moved about usually with assurance that the actual pattern of activity is neither moving to other loci or significantly changing in any other characteristic.

### *Regional responses*

In the case of both photic and electrical stimuli the cortical responses of greatest amplitude are found in the mid-dorsal region of the lateral gyrus. This includes a region where the characteristic striate architecture is well developed and a region where cell stains show a high population density of the large cells of layers II and III. The gyrus compositus or post-lateral gyrus rarely produces surface positive waves of an amplitude comparable with those recorded from the mid-dorsal region of the lateral gyrus. This gyrus appears to have fewer large cells in layers II and III and the strata appear to be thicker. Further, factors bearing on this peculiarity of the difference in response amplitudes will be discussed by one of the authors in a paper dealing with the problem of localization (see also 22, 23).

On the lateral gyrus the anterior limits of the electrical response, and also of the geniculate projection as indicated by retrograde degeneration, suggest that geniculate projection extends over a greater area than indicated by the classical structural definition of the area striata. Admittedly the electrical reactions near the margin may represent intra-cortical transmission, but we do not believe they are physical artifacts. Retrograde cell de-

generation is subject to error when applied to this sort of problem because of the possibility of incidental injury or thrombus posterior to the region excised. The sections show no evidence of this, however, and the degenerated region in the lateral geniculate occurs where it should, according to the plan suggested by Minkowski (17), on the rostral and ventro-medially directed tip of the lateral geniculate. It cannot be stated that the definitely extra-striate response of the cat is not relayed through the geniculate. The accurate geniculo-striate relation must be examined more carefully. O'Leary and Bishop also found a somewhat similar situation in the rabbit (19) but concluded that the "parastriate" area there was really striate. Our area of potentials resembling primary projection reactions is somewhat larger than that reported by Gerard, Marshall, and Saul (19) but it agrees rather well with their data. Our area also more closely resembles the area defined by Polhak (20) than it does the area defined as striate by classical anatomical definition. More data on this will be presented in a forthcoming paper (24).

Part of the reactions on the suprasylvian gyrus have many characteristics of primary projection reactions, and part of them are obviously due to projection from the striate area. The fact that part of the suprasylvian reactions persist immediately after removal of both striate cortices and section of collicular bound pathways, indicates some projection either from the lateral geniculate or from possible nuclei near the geniculate. The failure of the suprasylvian reaction after retrograde degeneration of the geniculate, suggests that the geniculate is involved in some way which is not yet clear, possibly bifurcation of path, or that some other retrograde degeneration process has directly affected the suprasylvian or some relay nuclei involved.

This reaction is similar to the response produced in the medial ectosylvian surface by an auditory click stimulus. The areas in which the visual and auditory responses are obtained do, in fact, overlap in the medial and dorsal part of the ectosylvian gyrus, an area considered to be in the primary projection area of the auditory systems (1), but we have never observed maximum potentials from each at the same point in the ectosylvian, nor auditory responses of this type in the suprasylvian. Interaction between the auditory and visual responses has been tested in cases in which they clearly overlapped and, also, in separate loci on the ectosylvian, and none have been found. Neither stimulus conditions the response to the other in these preparations. Apparently the neurones are separate even in areas in which both responses are recorded from the same electrode. This means that the overlap is only apparent and not physiologically real, for, at least, the positive responses. This conclusion is suspicious and demands further inquiry.

#### SUMMARY

- 1 A brief photic stimulus evokes, under several types and stages of anesthesia, one or more (multiple) cortical responses. A single electrical stimulus applied to the optic nerve does not evoke a multiple response of the type which follows photic stimulus.

2 The responses are usually predominantly surface positive, positive phase first. In light anesthesia negative components are prominent.

3 Application of 0.1 per cent picrotoxin to the pia enhances the positive component about 25 per cent and either initiates a large negative component or greatly enhances one already present. The positive and negative components are obviously due to separate neural processes. The positive component is associated with ascending cortical processes. The negative component is associated with descending neural processes. With picrotoxin it can be demonstrated that the negative wave is associated with activation of association areas and tectal regions. Application of nembutal to the same pial surfaces abolishes the negative wave and the projected reaction.

4 The separateness of the positive and negative processes can also be shown by cross conditioning of the cortical mechanisms, wherein a photic stimulus applied to one eye is succeeded by an electric shock applied to the opposite optic nerve. The positive components evoked by the latter reaction are subnormal, but the negative component may be greatly facilitated.

5 Facilitation of the negative-wave-producing mechanisms may build up over a period of several hundred msec. This is strikingly shown by the multiple response, with or without picrotoxin. The third or fourth component of a positive multiple response may be followed by a definite negative wave. If the excitability of the negative wave mechanisms is raised by picrotoxin, or by decreasing anesthesia, the negative wave moves by steps to the first primary response.

6 Electrical shocks applied to the optic nerve evoke in the cortex three positive spikes of axon dimensions. These are followed by a slow positive wave, which in turn is typically followed by a negative wave. The amplitude of the negative wave is subject to the factors discussed above.

7 The first two spikes in the cortical record are very resistant to depression by chemicals or subnormality due to repeated activation at frequencies above 10 per sec.

8 Both photic excitation of the retina and electrical excitation of the optic nerve evoke primary responses over both striate and extra-striate (or peristriate) regions.

9 The extra-striate reactions in the suprasylvian are of two types. One type is obviously due to activation of association pathways from the striate. The other exhibits characteristics of the primary projection reactions and appears to be relayed from the lateral geniculate or nuclei close to the latter.

10 It is possible to assign to particular cortical layers the components of the response to electrical stimulation of the optic nerve.

11 The predominant negativity of the photic cortical response in some cats cannot yet be adequately explained.

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pins were cut off below the skin and, if necessary, a single suture was used to close each wound. Figure 1 shows a roentgenogram of a limb with the pins in place. The early operations were performed with the aid of x-rays and a fluoroscopic screen but this was later deemed unnecessary. The limbs were examined frequently and if fixation was incomplete this was remedied at once by inserting a new pin. Operating instruments and the pins were kept in alcohol prior to use and although no other antiseptic precautions were taken very few infections were encountered.

Pinning of the joints involved by the muscle being investigated was found to be a most satisfactory method of immobilization and was employed in most of our experiments. Clin-



FIG 1 Roentgenogram of fixed limb with pins in place

ical literature contains many suggestions that joint damage produces trophic effects on muscle not attributable to simple disuse. Contrary to this belief, however, a small series of animals with cast immobilization showed atrophy which was quantitatively similar to that produced by pinning.

In every experiment a large group of animals was employed and representatives taken at random for testing as required. As a rule the muscles were tested for fibrillation by the electrical method (14) and their acetylcholine sensitivity evaluated subsequently (10). This latter was accomplished by injecting 0.5 cc. of aqueous acetylcholine bromide solution into the exposed abdominal aorta or into each iliac artery, using a No. 32 needle and a tuberculin syringe. The concentration of the solution was increased with each injection until the sensitivity of both normal and fixed muscles was determined. The dilutions used are indicated on Fig. 3. The so-called full-strength solution contained 10.0 mg. of acetylcholine bromide per cc. The volume of each injection and the rate of injection were kept constant. Reaction of the muscles was recorded simultaneously on a smoked drum by means of isometric levers connected to the Achilles tendons which had been cut free from the foot.

# THE EFFECT OF SKELETAL FIXATION ON SKELETAL MUSCLE\*

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MUSCULAR inactivity resulting from destruction of the motor nerve is accompanied by a number of well known but little understood phenomena. Of these atrophy, fibrillation and hypersensitivity to acetylcholine and potassium are the most outstanding. Cutting the spinal cord also results in cessation of voluntary muscular contraction. Muscles thus affected show no fibrillation but do exhibit an atrophy which is, in the initial stages, as marked as that due to section of the motor nerve (16). Hypersensitivity to acetylcholine is also present but is less pronounced than in the case of the fibrillating "denervated" muscles.

Both upper and lower motor neurone lesions result in a marked decrease in the activities of the muscles involved. A precise evaluation of the effect of immobilization alone on muscles with completely intact motor and sensory nerve pathways is attempted in the present experiments.

The disuse atrophy of skeletal muscle has long been recognized clinically. In 1776 John Hunter (6) mentioned and in 1854 James Paget (11) described such atrophy associated with pathological conditions of the joints.

Experimental disuse atrophy has been brought about by tenotomy (13, 9, 8, 2, 18) and by cast fixation (5, 7, 8, 17, 1). The former method is accompanied by a bizarre contracture (2, 18) and the latter may be complicated by the pressure of the cast on the muscles and blood vessels and the "weight-bearing" which is possible, to some degree, in most casts (17). The so-called disuse atrophy produced by isolation of the section of the spinal cord supplying nerve fibres to a muscle (18, 4, 12) may be complicated by other factors as a result of severance of nerve pathways to the muscle.

The object of the present experiments was to test the effect of a method of immobilization which would not be open to these criticisms and to follow the course of any resulting disuse atrophy in an experimental animal in which the time-course of atrophy due to other causes had been investigated.

## METHOD

Rats weighing from 175–250 g were used. Light ether anesthesia was employed during all operative and experimental procedures. The knee and ankle joints of the limb on one side (different sides in alternate animals) were fixed with steel pins. The object of the fixation was to prevent skeletal movement due to contraction of the gastrocnemius-soleus group of muscles and to maintain the bones in such a position that these muscles would not be stretched. In fixing the ankle joint the foot was placed at right angles to the lower leg and a pin was forced up through the bones of the foot into the tibia or the perosteum and connective tissue on the anterior aspect of this bone. The knee was fixed with the tibia at right angles to the femur by a pin forced through the knee joint into the tibia. The

\* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

ment (a few animals, not shown in Fig 1, were carried on up to 41 days) in every case in which the fixation remained adequate

No fibrillation was observed at any time in the muscles involved by the skeletal fixation. These muscles, as shown in Fig 3, demonstrated a marked increase in sensitivity to intra-arterially injected acetylcholine as compared with either the muscle on the normal side or with muscles in completely

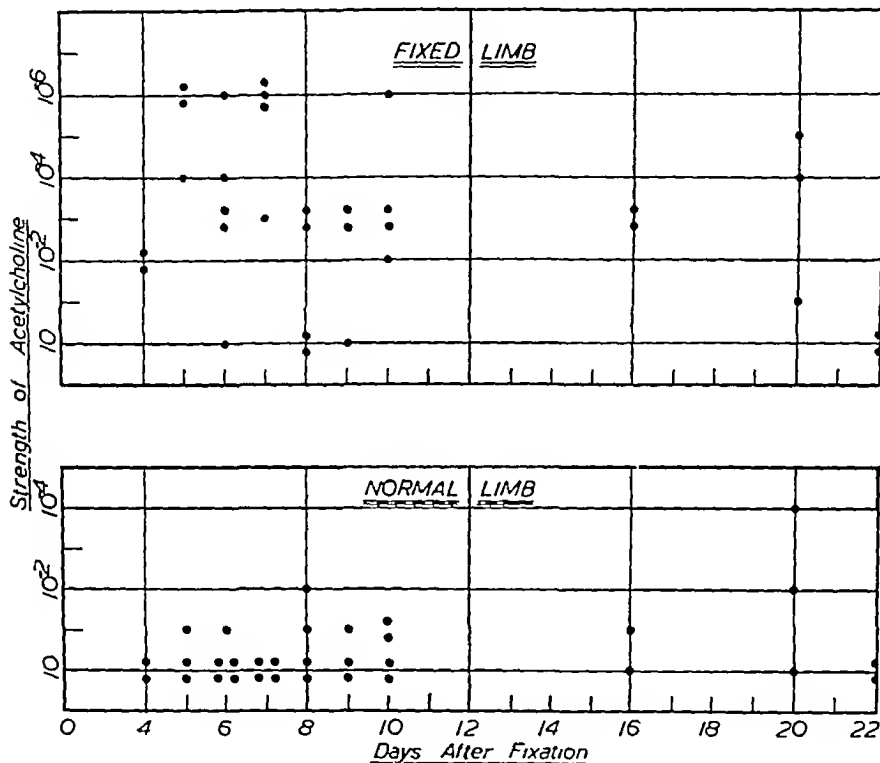


FIG 3 Strength of acetylcholine required to elicit a response from the gastrocnemius-soleus group of muscles in fixed (upper diagram) and normal (lower diagram) limbs. The "full-strength" solution, indicated as 10, is such that 5.0 mg of acetylcholine bromide are injected in the 0.5 cc dose given. The other strengths contain proportional amounts as indicated and are administered in the same volume. Each animal sacrificed contributes one point to each diagram.

normal animals. The muscles involved by skeletal fixation did not show as marked an increase in acetylcholine sensitivity as do muscles deprived of their lower motor neurone (16). The increase in sensitivity reached a maximum about 10 days after the operation and thereafter the sensitivity tended to return towards normal although the atrophy was maintained.

#### DISCUSSION AND CONCLUSIONS

The outstanding features associated with denervated muscle are the fibrillary activity, the atrophy, and the hypersensitivity to intra-arterially

In most experiments the motor nerves to the muscles were cut just before the test for acetylcholine sensitivity was made. Control experiments indicated that this procedure produced no immediate change in sensitivity. The elimination of reflex activity permitted a cleaner record being made. Before cutting the nerve neuromuscular continuity was tested by pinching the nerve or by electrical excitation of the nerve.

At autopsy the site of the pin at the knee was examined. Injury to large nerves or blood-vessels was never found. Finally, the gastrocnemius-soleus muscle groups from each side were carefully dissected out, blotted to remove any superficial moisture, and weighed immediately.

### RESULTS

Figure 2 indicates the degree of weight loss in the muscles affected by skeletal fixation. The weight of the "fixed" muscle (F) was divided by the weight

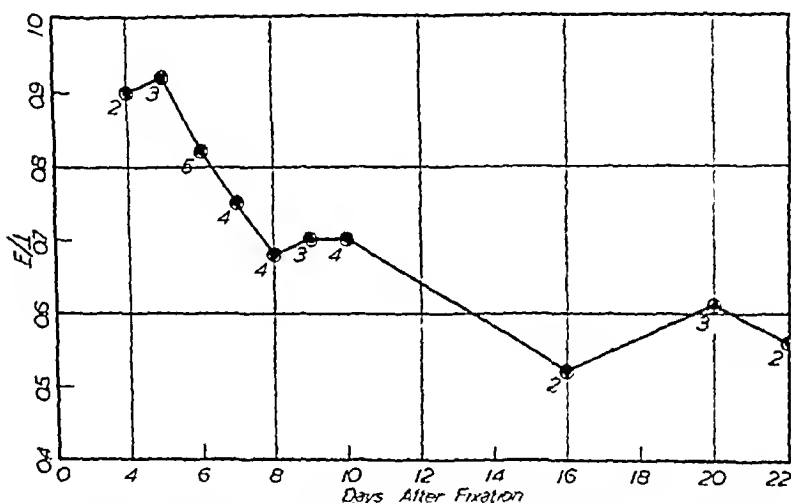


FIG 2 Change in weight of gastrocnemius-soleus muscle group following limb fixation. Atrophy is represented by the decimal fraction of the ratio  $F/I$  where  $F$  is the fresh wet weight of the muscles on the fixed side and  $I$  that of the corresponding muscles on the normal side. The figure adjacent to each point indicates the number of animals contributing to the average represented by the point.

of the muscle on the normal side ( $I$ ) and this quantity was plotted against the time in days after the operation.

The atrophy was very marked during the first 10 days and was about as great as that seen at the same time in muscles with the motor nerves cut (15) or in those deprived of upper motor neurone control (16). After the 10th to 14th day the atrophy no longer progressed but no significant reduction in atrophy was regularly observed. Whenever an animal was autopsied and showed a muscle weight indicating that regression of atrophy had occurred the joint-fixing pins were found to have become loose thus permitting some movement. The gastrocnemius muscles atrophied to from 50 to 60 per cent of the weight of the muscles on the control side in from 10 to 14 days. They then remained at or near this reduced weight for the duration of the experi-



ius-soleus group of muscles. The atrophy was initially as marked, but did not progress as far, as that seen in muscles paralyzed by the loss of their motor nerve supply. The atrophy produced was sustained for the duration of the experiment in all animals in which the fixation was maintained.

We should like to thank Professor C H Best for the kindly and helpful interest which he has shown in this work.

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injected acetylcholine and potassium. Fibrillation has never been observed by us in any immobilized muscle with an intact motor nerve. On the other hand, both atrophy and increased sensitivity to acetylcholine are seen in muscles with normal activity prevented by skeletal fixation or by section of the spinal cord (15, 16). It has been suggested by Denny-Brown and Pennybacker (3) that fibrillation is due to the hypersensitivity to acetylcholine and other experiments (10) have indicated that potassium may have a causative role. The fact that fibrillation is not seen in animals with the spinal cord cut or with the limb bones fixed may be due to the fact that the acetylcholine hypersensitivity induced by these procedures is never as great as that seen in denervated muscles.

Cutting the motor nerve to a muscle prevents all activity of the muscle except fibrillation. Fixing the parts of the skeleton which are normally moved by the muscle merely makes muscular contraction futile. Observations on the series of animals thus treated leads us to believe that the animal quickly adapts to the altered skeletal mechanics and ceases to employ the muscles rendered useless. These muscles may be contracted occasionally but, after the 2nd postoperative day, they are usually flaccid and apparently toneless. Cordotomy (16) prevented voluntary contraction of the muscles of the hind limbs. These muscles appeared to be relaxed and showed little tone most of the time. They were, however, capable of violent reflex contraction when properly stimulated.

All three types of experiment yield muscles which atrophy and show hypersensitivity to acetylcholine. Both these features are most marked and most permanent when the motor nerve is cut. Functionally one feature common to the three types of atrophic muscles is that they are not subjected to a normal amount of tension-producing activity. Fibrillation involves little increase in muscle tension. Lack of tension-producing activity may produce or contribute to the atrophy. Thompson (17) has shown that the marked atrophy obtained by splinting a rabbit's limb in a cast may be very largely prevented if the cast is so applied as to permit "weight-bearing." This observation may account for failure by others (8) to produce marked atrophy by simple immobilization. Fixation by pinning permits weight-bearing through the bones alone and prevents the muscles under consideration playing any part in such activity.

It is not clear what the connection is between the lack of tension-producing activity, the atrophy and the acetylcholine sensitivity. It is, however, an interesting and possibly an important observation that immobilizing a limb produces the same kind of changes in the muscles involved as does denervation of these muscles. This finding suggests that the methods of treating paralyzed muscles which involve immobilization should be re-examined and possibly modified.

#### SUMMARY

Skeletal fixation in rats was found to produce atrophy and hypersensitivity to intra-arterially injected acetylcholine in the involved gastrocnem-

changes are manifest. It has, however, been shown that, despite this decline of pressure, there is no general cardiovascular collapse until intraventricular block or cardiac arrest appear in the electrocardiogram. It has therefore been assumed that cerebral anoxemia due to circulatory failure did not occur with calcium and potassium injections until signs of serious derangement appeared in the electrocardiogram. It is quite possible that, with magnesium, some failure of cerebral circulation due to declining blood pressure may have preceded important electrocardiographic changes.

## RESULTS

(1) *Potassium chloride* No changes in the cortical electrogram could be demonstrated until intraventricular block or cardiac arrest had appeared.

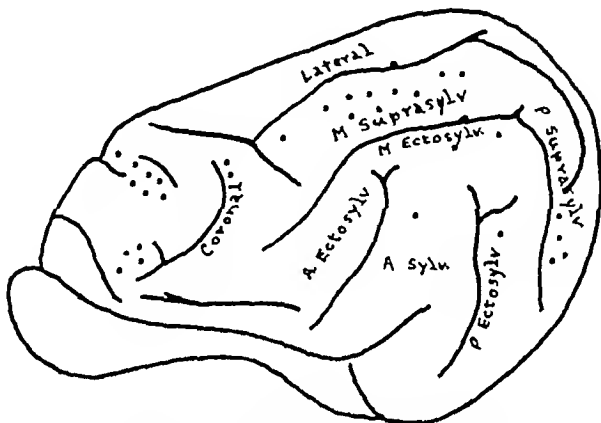


FIG 1 The heavy dots indicate points from which records were obtained in various experiments

After these cardiac changes were manifest, certain changes in the cortical electrogram did appear. These consisted of the development of large slow waves, sometimes preceded by temporary increase in the number of fast waves. The fast waves eventually disappeared, leaving only random slow waves, which persisted for some minutes after complete cardiac arrest.

(ii) *Calcium chloride* Usually little or no change could be detected as long as cardiac action remained fairly normal, although in one experiment there was a little slowing. After disruption or cessation of cardiac action the higher frequencies disappeared from the cortical electrogram, and the amplitude and frequency decreased progressively until only slow random baseline swings were demonstrable.

(iii) *Magnesium sulfate* Anoxemia due to respiratory failure was prevented by artificial respiration. In spite of this, the cortical electrogram was modified before any considerable change in the electrocardiogram was demonstrable (Fig 2). There was a marked initial slowing (Fig 2, 20 sec), followed by restoration of the initial frequency (Fig 2, 60 sec). Later, with the development of auriculo-ventricular and of intraventricular heart block (Fig 2, 120 sec), slowing of the cortical electrogram reappeared together with a disappearance of the rapid waves.

# INTRAVENOUS POTASSIUM, CALCIUM AND MAGNESIUM AND THE CORTICAL ELECTROGRAM OF THE CAT\*†

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ALTERATIONS in concentration of potassium and calcium in the blood stream may on occasion influence brain potentials (3). Since these ions affect both cardiac activity and blood pressure (7, 8, 13, 16), it is possible that some of the effects on the brain potentials which have been previously described are secondary to cardiovascular changes. In the present study an attempt is made to distinguish between those effects of potassium, calcium and magnesium ions on the cortical electrogram which are independent of cardiovascular disturbances and those which are regularly associated with them.

## MATERIAL AND METHODS

Sixteen cats under light nembutal anesthesia (35 mg per kg body weight, intraperitoneally) were used in these experiments. The left hemisphere was exposed and brain potentials recorded simultaneously from two or three areas, using lead solder electrodes 3 mm in diameter. A reference electrode was attached to the left ear. The areas of the brain on which the electrodes were placed in the various experiments are indicated by the heavy dots of Fig. 1. Although the simultaneous patterns from different areas of the brain were quite independent of one another, effects were not restricted to any one area of the brain. Simultaneous electrocardiograms were recorded from lead II. Grass amplifiers and ink writing undulators were employed to record the cortical electrograms and the electrocardiogram.

An isotonic solution of calcium chloride, potassium chloride, or magnesium sulfate was injected continuously into the right femoral vein. Five cats received injections of potassium chloride, five received calcium chloride and six were given magnesium sulfate. In two instances a control injection of isotonic saline was given prior to the main infusion. The rates of injection of each salt, usually about 15 cc per minute, was sufficiently rapid to induce the characteristic sequence of electrocardiographic changes known to be associated with a progressive increase in concentration of the ions in serum (7, 13, 16). Injections were continued until death from cardiac arrest. Artificial respiration was carried on throughout the experiments in which magnesium was injected. In previous experiments it has been found that, when potassium is given, the mean arterial blood pressure does not change significantly until the onset of marked intraventricular block or cardiac arrest (8). Blood pressure is unaffected by calcium injection until the moment of arrest or of ventricular fibrillation. Magnesium, on the other hand, produces a progressive decline in blood pressure beginning soon after the injection is begun, before any electrocardiographic

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‡ Honorary Fellow in Physiology, Yale University School of Medicine, May-September, 1939.

activities of the two brain regions represented by the two records are quite dissimilar. Both destruction of the brain tissue directly beneath the electrodes and application of water to the surface of the brain altered the pattern of the cortical electrogram indicating that the potential changes are not artifacts. Other control experiments demonstrated that they did not depend on the character of the electrodes or of the amplifiers themselves. During

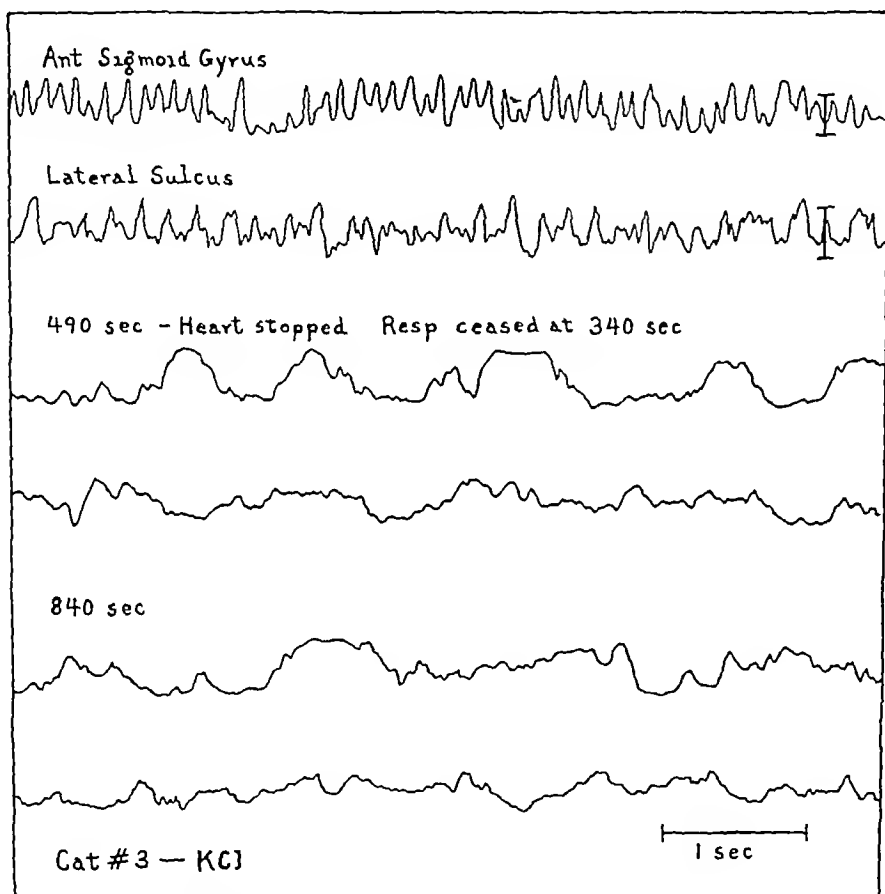


FIG 3 Cortical electrograms prior to infusion of potassium chloride solution (top records) and at intervals after cardiac arrest and respiratory arrest. The height of I corresponds to  $50\mu\text{V}$ .

this period of post-mortem electrical activity, pinching the foot, moving the paw, or pricking the footpad of the cat failed to evoke any response in the cortical electrogram.

#### DISCUSSION

Potassium and calcium, under the conditions of these experiments, exerted no distinct effect on the cortical electrogram until development of

Synchrony between electrocardiogram and cortical electrogram appeared in the experiment illustrated in Fig 2. It developed first at 90 sec in one brain area and at 110 in the other. By 150 seconds it had disappeared from the first region. This phenomenon has been described previously by Gerard, Marshall and Saul, who suggest that it may be due to local pulsations in the cerebral vessels (5).

(iv) *Sodium chloride* In two experiments 30 cc of isotonic saline were

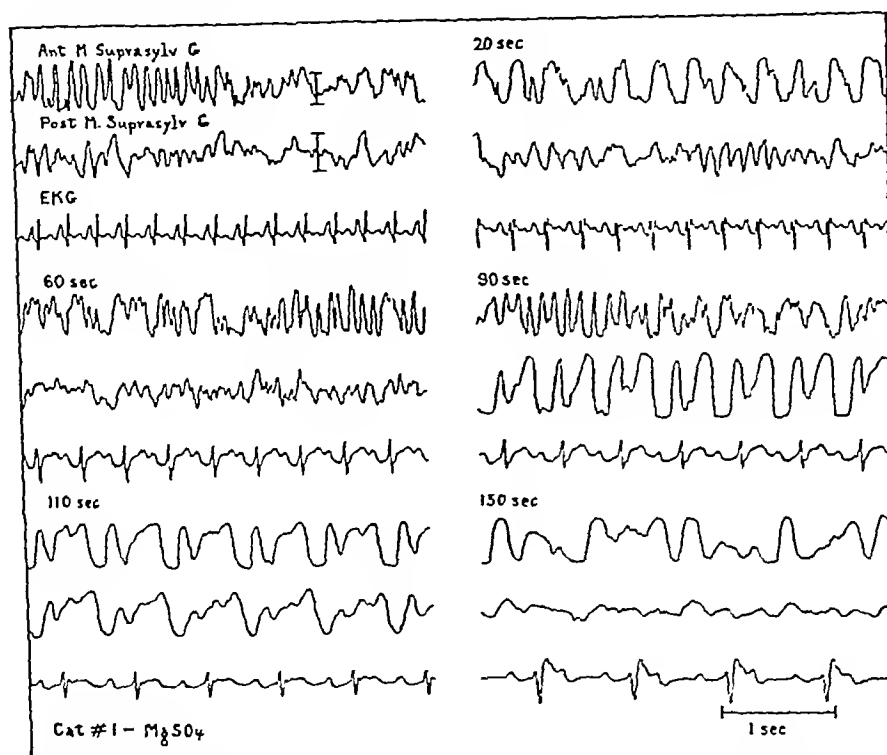


FIG 2 Cortical electrograms and electrocardiogram at various times during the continuous intravenous injection of magnesium sulfate in the cat. The height of I corresponds to  $50\mu\text{V}$ .

injected prior to infusion with magnesium sulfate. The rate of injection was the same as that in the other experiments. Neither the cortical electrogram nor the electrocardiogram was influenced by this procedure.

(v) *Post-mortem potential changes* In these experiments potential changes in the brain continued for at least 15 minutes after complete respiratory and cardiac arrest, irrespective of the particular ion responsible. This persistence of electrical activity is shown in Fig 3. In this experiment potassium had been injected until cardiac arrest had occurred. It can be seen that the random, slow potential changes 6 minutes after cardiac arrest are indistinguishable from those being recorded at the time of cardiac arrest. The electrical

the cat were followed continuously during the intravenous injection of salts of potassium, calcium and magnesium

2 Potassium and calcium produced no changes in the cortical electrogram until the development of intraventricular block or of cardiac arrest. Subsequently slowing of the cortical electrogram developed

3 Magnesium produced transient periods of slowing before pathological changes appeared in the electrocardiogram. A secondary slowing of frequency, much like that following cardiac arrest due to potassium or calcium, appeared after the development of intraventricular block

4 Brain potentials persisted for at least fifteen minutes after complete respiratory and cardiac failure

5 In concentrations tolerated by the intact cat, potassium and calcium are without demonstrable effect on the cortical electrogram while magnesium does have some effects

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terminal cardiac failure The anoxemia resulting from the failing circulation may well account in a large measure for the cortical electrographic changes observed during the injection of these ions The effects of magnesium are more difficult to interpret The early, transient changes in the cortical electrogram appear with amounts of magnesium well within the limits of cardiac tolerance, but nevertheless sufficient to depress the blood pressure These early effects might therefore be due either to a direct effect of magnesium on the brain potentials, or they might be secondary to the fall in blood pressure The fact that magnesium, in concentrations within physiological limits, may directly affect the central nervous system sufficiently to produce surgical anesthesia, is consistent with a direct effect upon brain potentials, but from our experiments there is no way of deciding which explanation is the correct one The secondary slowing of the cortical electrogram associated with electrocardiographic changes is comparable to the terminal effects observed with potassium and with calcium

During the course of this study the coronal, the anterior and posterior sigmoid, the middle suprasylvian and the middle and posterior ectosylvian gyri were explored The electrical activity from these different regions exhibited a high degree of independence (see Fig 2) Relative independence of various brain areas under different experimental conditions has previously been described (11, 14)

Anoxia alone, produced in a variety of ways, causes complete disappearance of brain potentials in the cat within one minute (1, 2, 5, 9, 12, 14, 15) The persistence of electrical activity in the central nervous system for some minutes after cardiac arrest was therefore rather unexpected The reasons for this unusual persistence of brain potentials are still obscure

Our observation that potassium has little demonstrable effect on brain potentials before its cardiac effects are manifest seems inconsistent with a statement by Emmens and Marks that potassium chloride, injected intravenously or intraperitoneally in mice, kills by an action on the central nervous system (4) They base this conclusion on the statement that death in their experiments occurs with convulsions and evident respiratory distress while the heart continues to beat We have repeated their experiments, in addition obtaining continuous electrocardiographic records until death In our experiments a period of cardiac arrest or of marked intraventricular block regularly preceded the convulsions and respiratory distress Electrocardiographic complexes, presumably corresponding to mechanical ventricular beats, were occasionally observed after respiratory failure These should not, however, be considered evidence of an adequate circulation Since the cardiac changes were sufficiently severe of themselves to be responsible for death, it seems unnecessary to postulate an additional effect upon the central nervous system We are thus unable to confirm their conclusion that the heart is not the primary cause of death

#### SUMMARY AND CONCLUSIONS

- 1 The cortical electrogram and the simultaneous electrocardiogram of



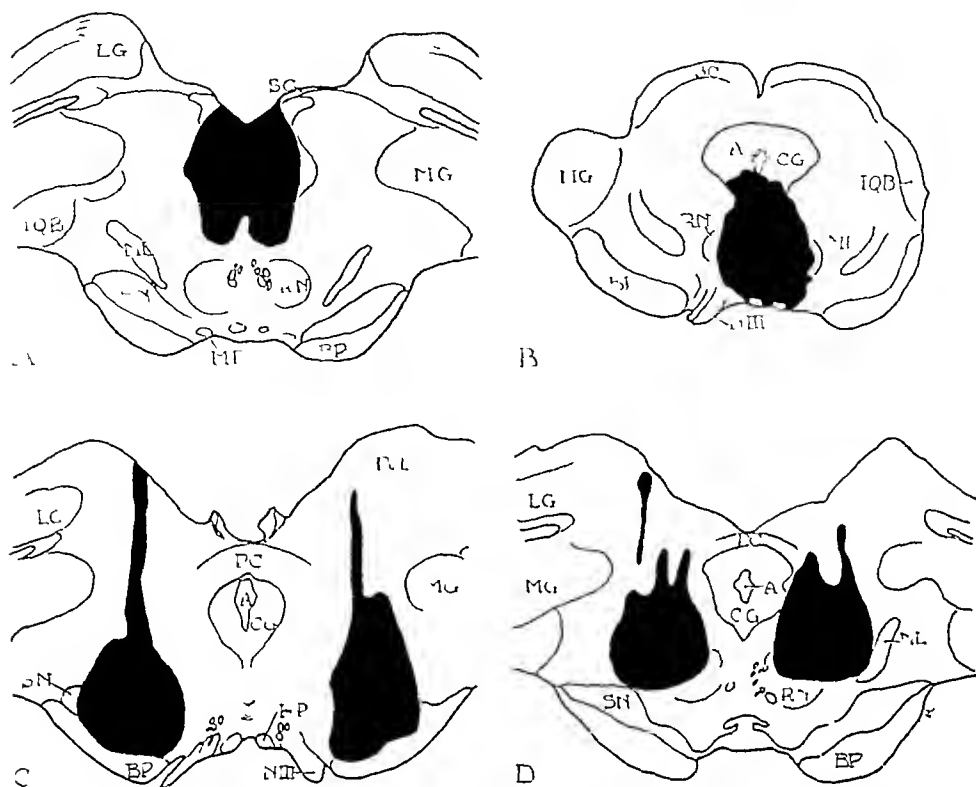


FIG 1 A-D Levels through the anterior portion of the midbrain showing the widest extent of the lesions in Cats 1-4, respectively Abbreviations for all figures are as follows

A aqueduct  
BP basis pedunculi  
BrP brachium pontis  
CG central grey  
HP habenulopeduncular tract  
IC inferior colliculus  
IQB inferior quadrigeminal brachium  
LF medial longitudinal fasciculus  
LG lateral geniculate body  
MG medial geniculate body  
ML medial lemniscus  
MP mammillary peduncle  
NMV fifth motor nucleus

NIII third nerve  
NVI sixth nerve  
NVIII eighth nerve  
P pyramid  
PC posterior commissure  
PO pons  
Pul pulvinar  
RN red nucleus  
SC superior colliculus  
SO superior olive  
SN substantia nigra  
Trap trapezoid body

hours in an icebox and exhibited good piloerection and shivering. When its body temperature was raised in a hotbox to  $102.5^{\circ}$ , the animal panted at a respiratory rate of 240 per min and showed pronounced sweating on all footpads.

In cat 2, a lesion destroyed the ventral part of the central grey and the medial region between it and the base of the brain (Fig 1B). During a 3 hour period in an icebox, 3 weeks postoperatively, this animal defended itself normally against cold, exhibiting marked piloerection and some shiver-

# THERMOREGULATORY PATHWAYS IN THE CAT BRAIN STEM\*

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THE HYPOTHALAMIC regulation of body temperature (4) is effected by little known pathways which descend through the brain stem and spinal cord to innervate the somatic and autonomic effectors concerned in this activity. Some information on the brain stem distribution of these pathways has been provided by Keller (2) and Blair and Keller (1), who have studied the temperature regulation of cats and dogs with chronic section of parts of the mid-brain and pons. They have found that heat maintenance activity can be entirely eliminated by transverse section of the medial quarter segments of the cephalic midbrain, while at the pontile level heat-loss activity is eliminated by sections involving only the lateral portions of the brain stem.

The present report is concerned with a further study of thermoregulatory pathways in the brain stem of the cat after more restricted lesions made with the Horsley-Clarke technique.

## METHOD

Using the Horsley-Clarke technique (3) electrolytic lesions were produced in a series of cats, and 2 to 5 weeks after operation, the animal's temperature regulation was examined during a 3-hour period in an icebox at 34°, and in a hotbox at 120°, and compared with the results of preoperative tests. All temperatures are in degrees Fahrenheit, and all animal temperatures were taken rectally. At autopsy the position and extent of the lesion were verified microscopically.

## LESIONS OF THE ANTERIOR MIDBRAIN

At the level of the anterior part of the midbrain, lesions in different animals destroyed the tectum and parts of the central and tegmental regions.

*Tectal region* Aspiration of the tectum of the superior colliculus, except for its most lateral portions, produced no impairment in temperature regulation. A tectumectomized cat, tested 2 weeks after operation, elevated its body temperature slightly during a 3 hour period in an icebox, and showed typical piloerection and shivering. When its body temperature was raised in a hotbox to 103.9° (preoperative threshold, 104.1°), it panted with a respiratory rate of 250 per min, showed good sweating on all footpads, and exhibited evident dilatation of the ear vessels.

*Central region* In cat 1, the lesion shown in Fig. 1A interrupted the central grey matter and the periventricular paths contained within it, yet the animal preserved perfectly normal regulation against heat and cold. Four weeks after operation it kept its temperature at a normal level during 3

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Another animal, in which smaller lateral lesions destroyed the periphery of the brain stem with irregular softening extending into the tegmentum, exhibited a normal defense against cold. Three weeks postoperatively, the body temperature remained at  $102.4^{\circ}$  during 3 hours in an icebox, and good

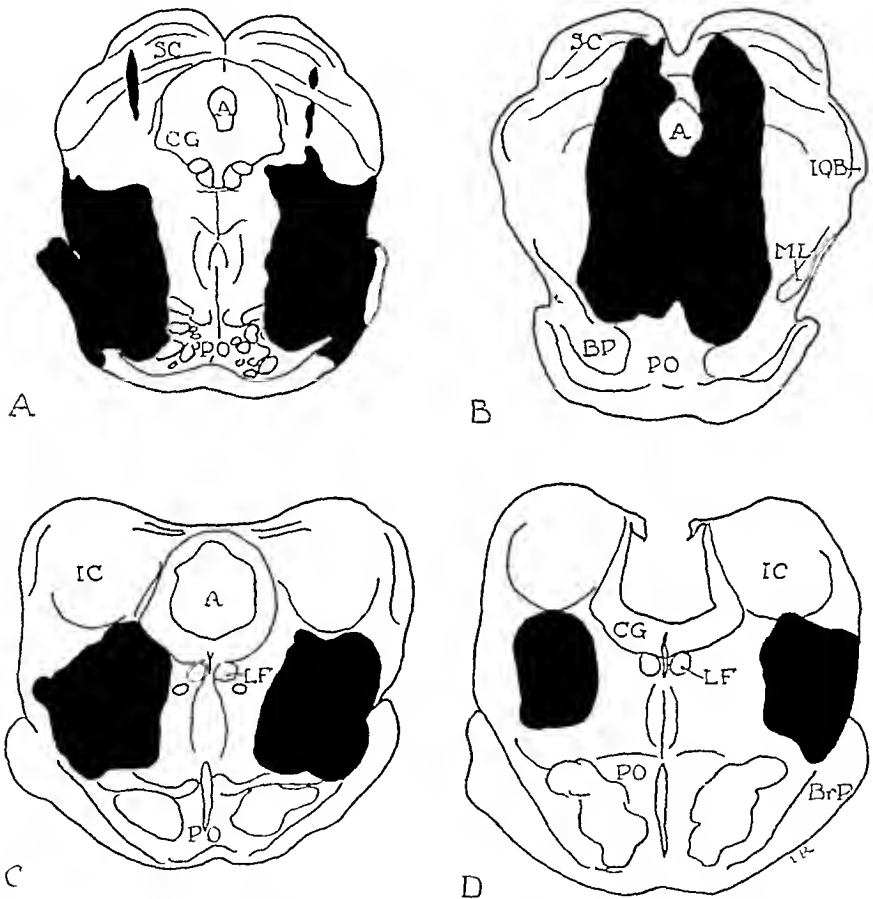


FIG 2 A-D Levels through the caudal midbrain showing the widest extent of the lesions in Cats 5-8, respectively

piloerection and some shivering occurred. Elevating this animal's temperature to  $107^{\circ}$  in a hotbox resulted in slight sweating and a polypnea of 180 per min with poor panting movements.

*Medial region.* Cat 6 with destruction of the entire medial part of the caudal midbrain (Fig 2B), when tested 2 weeks postoperatively, exhibited a slight fall in body temperature to  $99^{\circ}$  during a 3 hour period in an icebox, but shivered violently and showed moderate piloerection in this situation. When its body temperature was elevated to  $107^{\circ}$  in a hotbox it sweated pro-

ing In the hot box it sweated a little and panted between rectal temperatures of  $104^{\circ}$  and  $106^{\circ}$  (preoperative threshold,  $104.4^{\circ}$ ) Panting was irregular and spasmodic, however, and the respiratory rate did not increase above 180, whereas the animal's preoperative panting rate was 240 per min

*Tegmental region* Cat 3 with the bilateral lesions of the ventral tegmentum, substantia nigra and basis pedunculi shown in Fig 1C, when tested 3 weeks postoperatively, maintained its body temperature during a 3 hour period in an icebox and exhibited good piloerection and slight shivering In a hotbox it showed pronounced sweating on the footpads and panted, but at a higher threshold and with a slower respiratory rate than in the preoperative test

Cat 4 with bilateral destruction of more dorsal parts of the tegmentum (Fig 1D) raised its body temperature a little during a 3 hour period in an icebox and showed definite piloerection and slight shivering When the animal's temperature was elevated to  $106^{\circ}$  in a hotbox, its respiratory rate increased to 100 per min but no panting occurred There was no sweat on any pad, and the animal felt cool as though no vasodilatation had occurred Two other cats with large lesions placed more laterally in the tegmental region exhibited normal temperature regulation, except for an elevated threshold to panting in one

The results at the level of the anterior midbrain indicate either that the correct distribution of lesions for destroying the heat-conservation pathways was not attained, or that these pathways are so widely distributed at this level as to be only partially affected by lesions of the size produced, for no evident impairment in heat conservation could be detected in any of the cases An impairment in heat-loss activity, indicated by an elevated threshold for initiation and by a reduction in the total response, was greatest after the tegmental lesions in cat 4 In this case, some polypnea occurred but neither panting movements nor sweating were evoked on raising the body temperature to  $106^{\circ}$  A similar tegmental distribution of heat-loss pathways was found at more caudal levels

#### LESIONS OF THE CAUDAL MIDBRAIN

More pronounced impairment in temperature regulation followed lesions in the caudal midbrain

*Lateral region* Cat 5 with the large lateral lesions shown in Fig 2A, though its temperature was normal in a warmer environment, ran subnormal temperatures of  $96-97^{\circ}$  when the room temperature was  $71-72^{\circ}$ , and on these occasions no piloerection could be observed but periods of shivering were noted During 3 hours in an icebox, 3 weeks postoperatively, its temperature fell to  $95^{\circ}$  and though shivering was definite no clearcut piloerection could be observed An even more pronounced deficit in heat-loss function was found When the animal's temperature was elevated to  $107^{\circ}$  in a hotbox, the respiratory rate remained at 36 per min, and there was no panting or sweating

### LESIONS AT THE PONTILE LEVEL

The lesion in cat 9 interrupted all periventricular connections passing backward from the caudal end of the cerebral aqueduct, and extended asymmetrically into the deeper parts of the neuraxis (Fig 3A) Four weeks after operation, the animal showed only a slight drop in body temperature from  $101.4^{\circ}$  to  $100.6^{\circ}$  during a 3 hour period in an icebox, and exhibited very good piloerection and fair shivering. When its temperature was elevated in

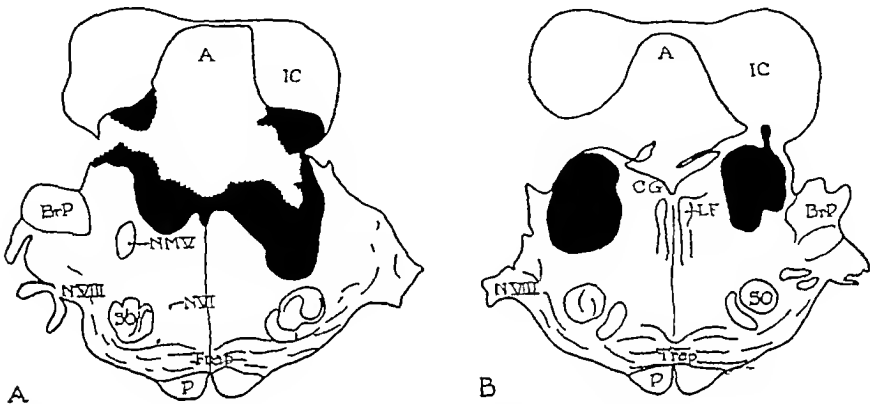


FIG 3 A, B Oblique sections at the pontile level showing the widest extent of the lesions in Cats 9 and 10

a hotbox to  $105.6^{\circ}$ , it showed a polypnea of 174 per min with a few panting movements when the mouth was opened, but no sweating occurred.

Cat 10 with lesions of the dorsolateral tegmentum shown in Fig 3B, exhibited a slight fall in body temperature from  $101.4^{\circ}$  to  $100.5^{\circ}$  when tested for 3 hours in an icebox, 5 weeks postoperatively. Good piloerection occurred but there was no discernible shivering, though the animal shivered well in its preoperative test. When heated to  $106^{\circ}$  in a hotbox, the animal's respiratory rate increased to 96 per min, and slight sweating was seen on the right forepad, but no panting occurred.

At this as at more anterior levels, the impairment in heat-loss function appears the result of interrupting pathways concentrated in the intermediate or lateral part of the dorsal tegmentum. Interruption of periventricular connections in cat 9, did not impair heat-conservation activity sufficiently to cause any significant abnormality in the animal's regulation against cold. The dissociation in heat-conservation function encountered after the lateral lesions in cat 10, in which shivering was abolished while piloerection remained elicitable, suggests that the different heat-conservation activities, like the different heat-loss activities, are effected by distinct anatomical connections rather than by collaterals from a single pathway.

The presence of piloerection in response to cold, and also in response to the presence of dogs, in cat 9 and 10 must mean that the tegmental injury

sely on the footpads, but did not pant and the respiratory rate increased only from 42 to 66 per min. The next 2 cases indicate that the tegmental rather than the central extent of the injury in this animal was responsible for the heat-loss deficit.

*Tegmental region.* Cat 7 with bilateral tegmental lesions, sparing the central grey matter and the paramedian region below it (Fig. 2C), was tested 3 weeks postoperatively. During 3 hours in an icebox it showed a slight fall in body temperature from 101.8° to 100.8°. It shivered well but piloerection was only slight. When its temperature was elevated to 107° in a hotbox, the respiratory rate remained at 40 per min and no panting or sweating occurred.

In cat 8, a smaller tegmental lesion (Fig. 2D) produced just as severe an impairment in heat-loss function. Three weeks postoperatively, when its body temperature was elevated to 107° in a hotbox, the respiratory rate was 42 per min and there was no panting or sweating. During a 3 hour period in an icebox, its temperature fell from 102.8° to 100.3°, then rose and remained at 101°. It shivered markedly and showed intense piloerection. Smaller and slightly more medially placed lesions of the dorsal tegmentum in another animal were followed by normal temperature regulation except for a slight elevation in the threshold for panting.

The impairment of heat-loss function encountered after lesions of the caudal midbrain appears to be the result of interrupting tegmental pathways, for it was just as extreme following lesions confined to the tegmentum as in cases with injury to adjacent parts. The heat-loss pathways concerned appear to be more numerous in the intermediate tegmental region than in the paramedian part, for intermediately placed lesions (cat 7 and 8), sparing the paramedian region, caused even greater impairment than did more medial lesions which included it (cat 6). The two components of heat-loss activity, facio-respiratory alteration and sweating, would appear to be activated by distinct structural connections, rather than by collaterals from a single pathway, for the lesion in cat 6 dissociated the two, an elevation of body temperature in this animal resulted in profuse sweating but only questionable polypnea and no panting.

Similarly, heat-loss and heat-conservation activities may be dissociated by lesions at this level, as has previously been shown by Keller (2). Heat conservation was most markedly impaired after the laterally placed lesions in cat 5, suggesting a concentration of connections for this activity in the lateral part but not in the periphery of the caudal midbrain. This animal was still able to shiver, however, and maintained its body temperature nearer the normal level than do animals in which the heat-conservation mechanism is entirely destroyed at the hypothalamic level. It is clear, therefore, that the heat-conservation pathway is not confined to the lateral region, though seemingly better represented there than in the central region, the extensive destruction of which in cat 6 resulted in only a minor impairment in this function.



in these cases was insufficient to interrupt the pilomotor pathway which Walker (5) has located at this level in the dorsolateral tegmentum adjacent to the brachium conjunctivum

### SUMMARY

The brain stem pathways subserving temperature regulation in the cat have been investigated by examining the animal's regulation to a hot or cold environment, 2 to 5 weeks after producing bilateral lesions at anterior and caudal midbrain and at pontile levels

Pathways subserving heat-loss functions appear to be concentrated in the intermediate and lateral part of the dorsal tegmentum at each of the levels studied

Pathways subserving heat-conservation activities appear to display some concentration in the lateral as opposed to the central portion of the caudal midbrain and pons

Following appropriately situated lesions, heat-loss activities were in some cases abolished while heat-conservation activities were maintained. This dissociation clearly indicated the existence of dual heat regulating mechanisms, whose efferent pathways are sufficiently independent to be destroyed separately

In the case of both heat loss and heat conservation, the component autonomic and somatic activities, sweating and facio-respiratory alteration, and piloerection and shivering, have been observed to be dissociated following appropriate lesions. Apparently these different activities are mediated by distinct anatomical connections rather than by collaterals from single pathways

In general the results present additional evidence for the greater importance of descending tegmental as compared with descending periventricular connections in efferent conduction from the hypothalamus

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tion of pathways for shivering and piloerection in the lateral column, but do indicate that they are well represented elsewhere in the cord

In each of these 5 monkeys with lateral column lesions at the cervical level, sweating on exposure to heat was equal on the 2 feet and in one it was equal on the 2 hands. In the other 4, sweating was impaired on the contralateral palm. In one animal this was indicated only by a delayed appearance of sweat on the contralateral palm, in the others sweating on the contralateral palm was significantly reduced. In 2 cases the contralateral palm was also less flushed than the ipsilateral. These results indicate a significant but not exclusive representation of sweat pathways in the lateral column of the cervical cord, those for the arm being better represented than those for the leg. A similar vasodilator representation is also suggested. The crossed distribution of sweating and flushing, analogous to that seen after hemisection of the lower brain stem, indicates that the decussation in the heat-loss pathway is a spinal one, that for the arm component being situated below C 4.

In each of 3 monkeys, sections interrupted the lateral column at thoracic levels below the preganglionic outflow to the upper extremities, and in 2 of the cases the adjacent portion of the anterior column was also injured. In the latter animals, an impairment in piloerection and shivering in the ipsilateral flank and leg was noted, suggesting the representation of pathways for responses to cold in the anterior rather than the lateral column of the spinal cord. In all 3 cases an impairment in sweating on the contralateral foot was evident, and in the monkey with greater injury to the anterior column, sweating was abolished on the contralateral foot while profuse on the ipsilateral. Sweat pathways for the lower extremity are evidently located in both the lateral and anterolateral columns of the thoracic cord and cross below the midthoracic level.

#### SUMMARY

In the monkey, thermoregulatory pathways for sweatings are located in the lateral and anterolateral columns of the spinal cord and exert a completely or almost completely crossed influence. The crossing is a spinal one, located close to the level of preganglionic outflow.

Little evidence was encountered for the presence of pathways for piloerection and shivering in the lateral column of the spinal cord. These pathways appear to be situated in the anterior column, and are both crossed and uncrossed, the uncrossed component being the greater.

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# SPINAL DISTRIBUTION OF THERMOREGULATORY PATHWAYS IN THE MONKEY

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STUDIES OF descending thermoregulatory pathways in man have indicated their predominantly ipsilateral distribution in the lateral and anterolateral columns of the spinal cord (1, 2, 3, 4) Tests of temperature regulation in each of 10 monkeys with lower brain stem or spinal cord lesions indicate a distribution of thermoregulatory pathways in the monkey in part like and in part different from that in man

## METHOD

In 2 animals (*Macaca mulatta*) the lower brain stem was hemisected at the pontile level In 8 others unilateral lesions of the lateral or anterolateral column of the spinal cord were made at the cervical or thoracic level The animals were prepared for other purposes but 2 to 6 weeks after operation, they were tested in an icebox at 32°F or by confining their trunk and lower extremities in a hotbox at 122°F In each case the extent of the lesion was verified at autopsy by microscopic examination

## RESULTS

In the 2 cases with hemisection at the pontile level, an unexpected dissociation was encountered in the distribution of pathways subserving the responses to heat and cold On exposure to heat both monkeys sweated profusely on the side of hemisection, while sweating on the opposite side of the body was barely perceptible in one case and wholly absent in the second animal These results indicate that little or no crossing in the descending sweat pathway occurs above the level of the pons, but between the pons and the thoraco-lumbar outflow this pathway undergoes a complete or almost complete decussation

On exposure to cold, the 2 hemisected monkeys piloerected and shivered on both sides of the body In each case, however, piloerection was more marked on the intact side Shivering was more pronounced on this side in one case and appeared equal on the 2 sides in the second animal After the animals were removed from the icebox, shivering and piloerection relaxed and disappeared first on the side of hemisection, at a time when they still remained marked on the intact side These results indicate that the descending pathway for piloerection and shivering is both crossed and uncrossed, the uncrossed component being the greater

In each of 4 monkeys with sections of the spinal cord destroying the lateral funiculus at C 4, except for varying amounts of its most ventral part, piloerection and shivering were present on exposure to cold and equal in degree on the 2 sides of the body In a fifth case shivering was slightly reduced in the ipsilateral arm The results do not rule out a minor representa-

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Each experiment began with a determination of the threshold of aural microphonics for clicks and pure tones as recorded from the round window with a large silver-wire electrode. Animals shown by this test to be markedly less sensitive than normals almost invariably had suffered section of the cochlear blood vessels during the operation. If the round window responses were normal, the cochlear electrode was removed and the microelectrode introduced into the auditory nerve with a micromanipulator until large,

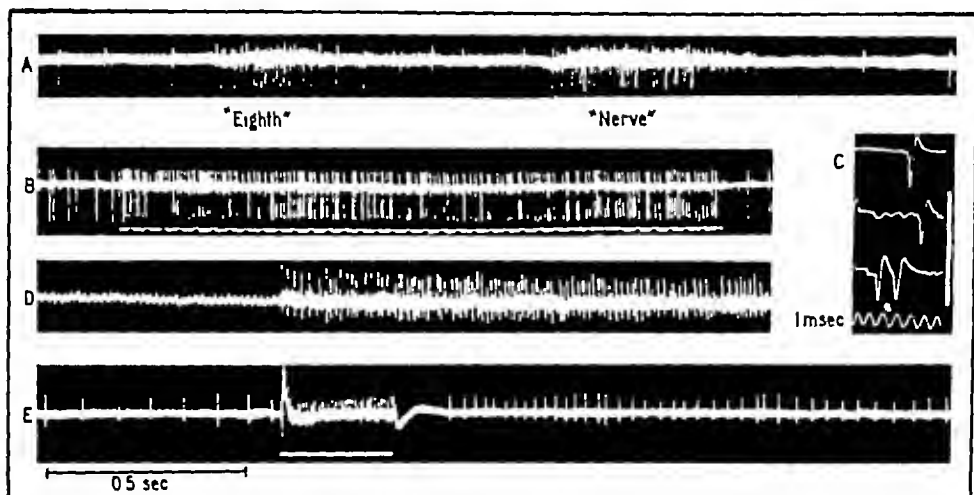


FIG. 1 Responses of single auditory-nerve fibers. In this and subsequent records a downward excursion indicates microelectrode negativity, the white line below a record marks duration of acoustic stimulation, and numbers in parenthesis indicate the characteristic frequency and minimal intensity for the fiber in question, reference level for intensity being 2 volts from oscillator. A, effect of the words "eighth nerve" upon the response of a fiber specifically sensitive to 2000 c p s. B, effect upon response of another fiber (17,100, -82 db) of a 17,100 c p s tone at -60 db. C, high-speed sweeps of the fiber shown in B. Upper sweep, no sound on, lower two sweeps, response to 17,100 c p s, -60 db. The time line is an oscillogram of a 1000 c p s tone. D, another fiber (7000 c p s, -90 db) responding to 7000 c p s, -68 db, in which no spontaneous activity like that shown in A and B occurred preceding onset of stimulus. E, example of the "silent period" and "after-discharge" following sound stimulation. This fiber (10,000 c p s, -84 db) was stimulated with 10,000 c p s, 0 db—a very intense tone.

simple, diphasic potentials registered on the tube face when a vocal sound (or a whistle or hiss) was made near the cat's ear. The experimenter then left the cat isolated in a quiet sound-deadened room, and presented subsequent sounds by remote control through the loudspeaker.

### RESULTS

*A General.* Figure 1 shows the type of response obtained from favorable preparations. In A, the words "eighth nerve" spoken in a conversational tone at a distance of about 20 feet from the cat elicited the response pictured. In B, acoustic stimulation caused an increase in rate of discharge of another isolated unit.

# THE RESPONSE OF SINGLE AUDITORY-NERVE FIBERS TO ACOUSTIC STIMULATION

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THE AIM OF the experiments described here was to study the response of single auditory-nerve fibers to acoustic stimulation delivered to the intact ear. Similar studies on other single afferent fibers have remarkably extended knowledge of the mode of action of diverse sensory mechanisms, while at the same time emphasizing their fundamental similarities. The present report describes the behavior of single auditory fibers and stresses similarities to other sensory nerves, in a subsequent paper we hope to relate these findings to a specific theory of action of the mammalian cochlea.

## METHOD

Young cats anesthetized with dial (0.75 cc per kilo) were used in these experiments. The postero-dorsal aspect of the auditory nerve was exposed by removing the lateral portion of the occipital bone where it meets the petrous bone. Bleeding from the sinus petrosus inferior was stopped by judicious cauterization or by Clotting Globulin.\*

A Ringer-filled glass micropipette with Ag-AgCl wire inserted as close as possible to the tip served as the active lead from the nerve. It was early established that pipettes with openings greater than  $5\mu$  do not allow isolation of the action potentials of single auditory fibers. The 3 to  $5\mu$  electrodes used have an impedance of about 1 megohm when tested on a resistance-capacity bridge between 0.6 and 2.5 kc. The microelectrode and the indifferent electrode (a silver plate in the neck muscles) led to a capacity-coupled amplifier (Grass) with an input impedance of about 8-10 megohms. Recording was done photographically from a cathode ray oscillograph. A neon bulb at the edge of the cathode-ray tube-face signalled the duration of presentation of sound.

Pure tones were ordinarily used as stimuli. A rubber hose conveyed the sound from a loudspeaker to the ear of the animal. The sound system differs in no important respect from that already described from this laboratory† (4). The tones were generated by a beat frequency oscillator having a range up to 40,000 c.p.s. (G.R. 713-B). Their intensity was controlled by an attenuator graduated in 2 db steps. Our reference level (0 db) is 2 V output from the oscillator. This level corresponds to a sound intensity delivered to the ear of the animal of approximately 100 db above human threshold at 2000 cycles.

\* We are indebted to the Lederle Laboratories for a generous supply of this effective hemostatic agent.

† For a complete description see J. E. Hawkins, Jr., "Electrophysiology of the Auditory Area of the Cerebral Cortex," a thesis on file at Widener Library, Cambridge, Mass.

jarred the preparation. The monophasic discharge had a high initial rate and relatively large amplitude. During the next few minutes both rate and amplitude progressively diminished until the discharge became lost in the random activity of the base-line.

The monophasic discharge just described has been interpreted as injury potential set up at the site of the electrode by its coming in contact with the fiber. The obliteration of the positive phase and increase in amplitude and duration of the negative phase, as well as the abrupt transition from diphasic

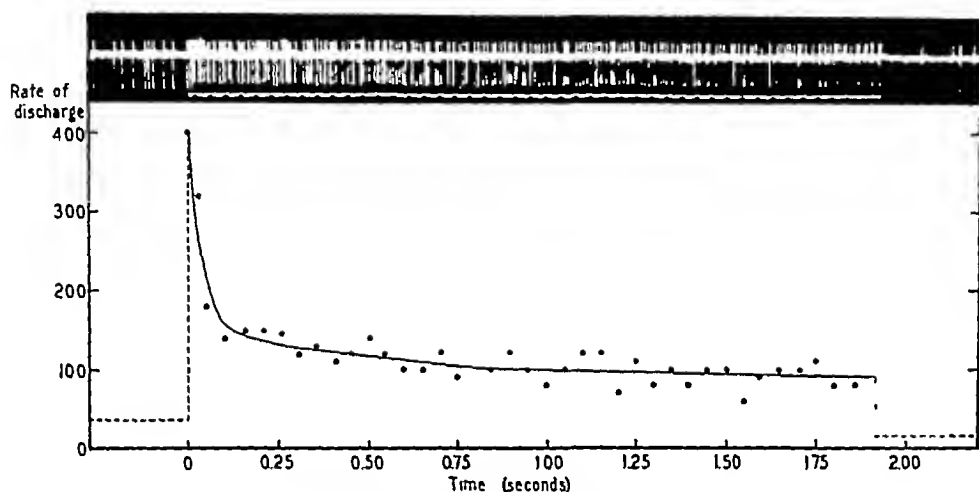


FIG. 3 Rate- and amplitude-adaptation by single auditory-nerve fiber (17,100 cps -82 db)

to monophasic discharge, support this interpretation. Figure 2 shows examples taken from the records.

Sound stimulation causes an increase in rate of monophasic discharge analogous to that described for the diphasic discharge (Fig. 2C, D). This probably means that even though the nerve is being excited by the contact of the electrode, with consequent passage of impulses toward the periphery, the end organ is still able to stimulate in the normal way. Such impulses as do arise, however, ascend only as far as the electrode.

The isolated auditory fiber may discharge in the absence of acoustic stimulation. Examples of this activity, which will be termed "spontaneous," are seen in Fig. 1A to C. More than half the fibers isolated reacted spontaneously, occasionally (Fig. 1D) one did not. Spontaneous activity appears to be common in sensory nerves (see 8, p. 572). In reading records from such fibers, excess discharge over the spontaneous rate may be taken as indicating response to the acoustic stimulation delivered by the experimenter.

A brief silent period during which spontaneous activity is absent often occurs upon cessation of stimulation (Fig. 1B, E). Following very intense

Evidence that the isolated unit is a single nerve fiber comes from the simplicity of the recorded electrical pattern (Fig 1C) The records consistently show an initial negative spike followed by a positive phase, and the amplitude and duration of the discharge remain practically constant in spite of variation of frequency and intensity of stimulation over a period of many hours In some records, it should be pointed out, the negative phase is reduced or absent (Fig 9)

Further support for the conclusion that electrical records from single fibers are here under consideration comes from the difficulties experienced in setting up an adequate preparation A smooth base-line broken by sharp

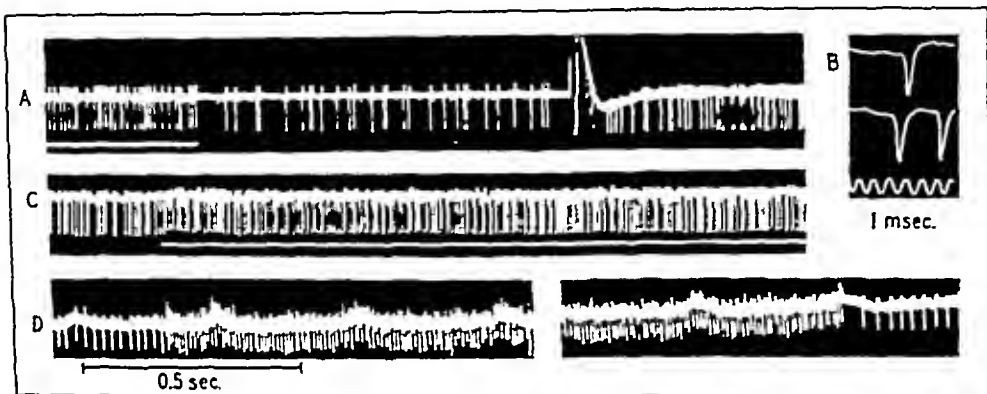


FIG 2 Responses of single auditory-nerve fibers A, B, and C, the same fiber pictured in Fig 1, B and C (17,100 c.p.s., -82 db) A, diphasic responses become monophasic after the artefact, which marks turning off of the camera motor B, sweeps of the monophasic response shown in A and C Compare with Fig 1C The time line is an oscillogram of a 1000 c.p.s. tone C, effect of 17,100 c.p.s., -68 db D, another fiber (4000 c.p.s., -90 db) in which stimulus of 4000 c.p.s., -80 db causes increase of monophasic response Note brief silent period at off

spikes when the sound goes on is not commonly seen Usually the electrode picks up numerous spikes of varying amplitude, giving a complicated picture obviously representing activity in many neural units Subsequent careful adjustment of the microelectrode often enhances the response of one unit while diminishing that of the others, which become merely annoying fluctuations on the base-line These manipulations, it should be noted, often increase or decrease the relative amplitude of the positive phase This fact is difficult to reconcile with the known properties of the nerve discharge unless it be assumed that the geometrical relation between very small microelectrodes and the active tissue determines in some important way the electrical pattern which will be recorded

On more than one occasion during the recording of single fiber potentials, the customary diphasic pattern suddenly changed to a monophasic negative discharge The phenomenon usually occurred during manipulation of the electrode or following some activity by the experimenter which could have

the intensity of the sound striking the ear. When intensity is fixed at a value just sufficient to cause minimal response, only a narrow band of sound frequencies excites a given auditory-nerve fiber. Thus we may assign to each fiber a characteristic frequency band and a particular minimal intensity, and refer to these as the "characteristic frequency" and the "minimal intensity" respectively. For example, a particular fiber increased its rate of discharge a just perceptible amount over the spontaneous rate (*i.e.*, it "began to respond") when sound frequencies near 2000 c p s at an intensity level of

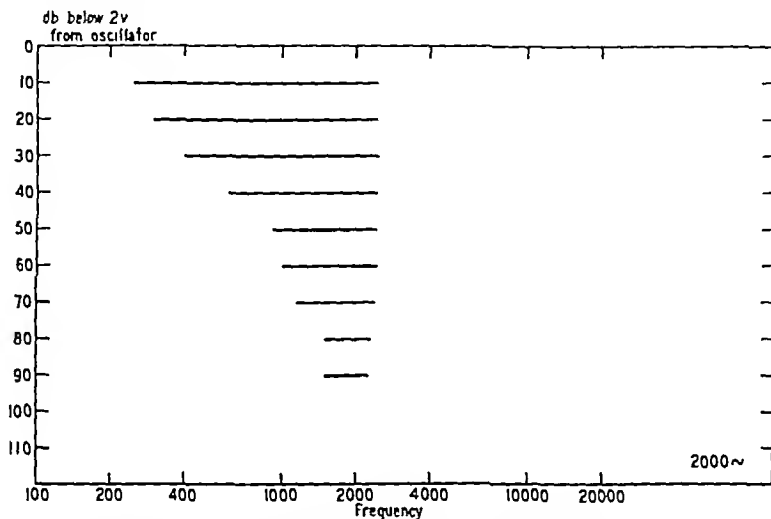


FIG. 5. Data used to construct the response area for an auditory fiber (2000 c p s, -100 db). At the intensity level given on the ordinate those frequencies covered by the horizontal line caused the fiber to respond. Note that as intensity increases (less attenuation) the range of stimulating frequencies is extended. The spread, however, is unsymmetrical.

100 db (below 2 V) were presented to the ear. At 102 db, all frequencies were ineffective. This fiber, therefore, had a "characteristic frequency" of 2000 c p s and a "minimal intensity" of -100 db, and it may hereafter be designated as "the 2000 c p s, -100 db" fiber.

Figure 4 shows the characteristic frequency and minimal intensity values for some forty fibers. It will be noted that no fibers with characteristic frequency below 420 c p s were isolated in spite of repeated attempts to find them. This may mean that the cat has no fibers specifically sensitive to frequencies below about 400 cycles, or that the standard operation used throughout this study does not favor their isolation.

How extensive is the range of frequencies which excite at minimal intensity? For a 700 cycle fiber the range was  $\pm 10$  cycles, *i.e.*, between 690 and 710 c p s. For a 7000 cycle fiber it was  $\pm$  about 100 c p s. Thus although in terms of cycles per second the bands are narrow for "low frequency" and broad for "high frequency" fibers, relatively they are about the same. Each

stimuli, the silent period may be succeeded by a period of marked acceleration of the spontaneous discharge (Fig 1E)

*B Adaptation* A decrease in the voltage output of the auditory nerve shortly after onset of sound stimulation was noted and termed "equihbation" by Derbyshire and Davis (4) They attributed this phenomenon to (1) decrease in the rate of discharge and (11) decrease in voltage output of each responding fiber We shall refer to these two factors as rate-adaptation and amplitude-adaptation, respectively Figure 3 shows that this explana-

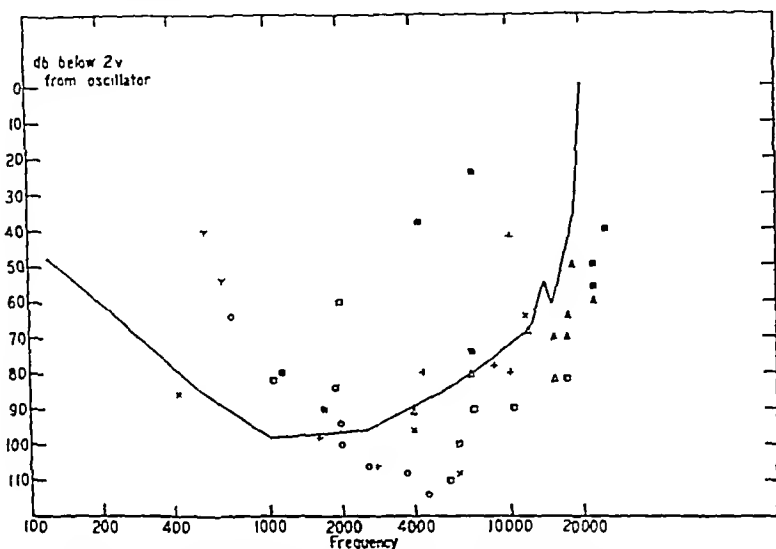


FIG 4 Frequencies and intensities at which minimal responses were obtained from single auditory-nerve fibers Each point marks the characteristic frequency and minimal intensity of one unit Solid line shows aural microphonic thresholds for one of the cats used in these experiments (nerves isolated from this cat are indicated by the open squares) Each symbol represents a different animal

tion, offered for data collected with a (large) co-axial electrode, is supported by the study of single fiber responses The rate of nerve discharge at the onset of stimulation is relatively high, but drops off with time, this type of adaptation in auditory fibers is like that occurring in other sensory fibers (1, p 23 ff) Furthermore, a diminution of spike height (voltage) also takes place in accord with observations that rapid discharge prevents complete functional recovery of the nerve fiber (7) As a general rule, both rate- and amplitude-adaptation can be expected to be substantially complete within a few tenths of a second after a fiber begins to respond (see Fig 1B, and 9) Some fibers, like that pictured in Fig 7, appear not to exhibit the adaptation phenomena

*C Minimal response of single fiber* Whether or not a single auditory-nerve fiber will respond to a pure tone depends upon both the frequency and



facts will be considered in detail later as evidence that larger and larger areas of the basilar membrane respond to increasingly more intense tones

A line connecting the ends of the frequency bands shown in Fig 5 encloses a roughly triangular area which contains each sound frequency and intensity capable of stimulating the given fiber. This area may be termed the "response area" for that nerve fiber, and each response area is unique. Figure 6 shows response areas for three fibers from one animal, where over-

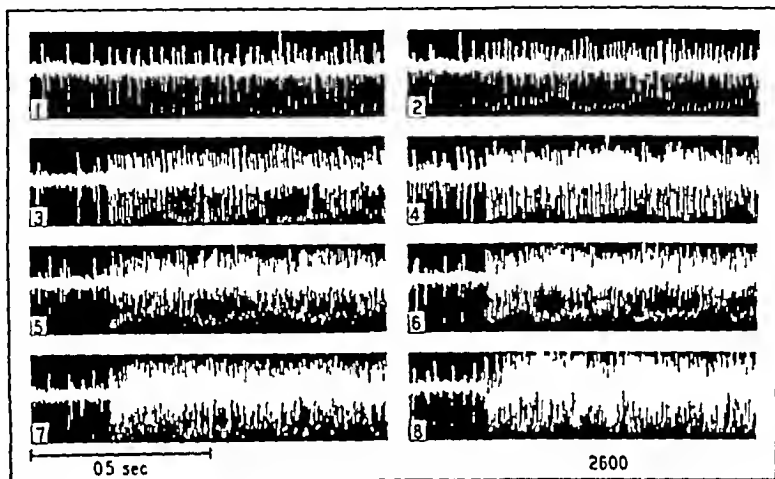


FIG 7 Frequency of nerve discharge as a function of sound intensity. This fiber (2600 cps, -110 db) behaves toward increase in intensity by increasing its rate of discharge. Intensity level at which above records were taken (in db) 1, -110, 2, -106, 3, -102, 4, -98, 5, -94, 6, -90, 7, -80, 8, -60. The gradual coarsening of the baseline as intensity is increased probably means that the microelectrode, although singling out one fiber fairly clearly, was picking up the responses of many fibers in the same tract.

lapping occurs, stimuli are defined which would have excited both (or all three) fibers.

The continuous "V"-shaped line which marks the lower boundary of each response area in Fig 6 may be thought of as the threshold curve for excitation of the corresponding fiber. This threshold excitation curve gives the intensity required to elicit a just perceptible increase in nervous activity at each frequency.

*D. Response of the single fiber as a function of sound intensity.* The rate of discharge of an isolated auditory fiber is determined by the frequency, the intensity, and the time after onset of the stimulating tone. How the fiber "equilibrates" or adapts with time has already been demonstrated (Fig 3), and how rate of discharge depends, at constant intensity, on sound frequency will be treated under "iso-intensity contours" (see below), this section will

fiber may therefore be said to "tune in" sharply to a specific and narrow region of the sound spectrum. This finding is of first importance in establishing the mode of action of the cochlea in hearing.

On comparing minimum intensities required for activation of nerve fibers and for the appearance of aural microphonics (Fig 4), two important points emerge. First, aural microphonic thresholds (measured at the round window) appear to be a poor index of the minimum sound intensity required for nerve stimulation. This is particularly true for high frequencies while no cochlear potentials above 20 kc can ordinarily be measured at the round

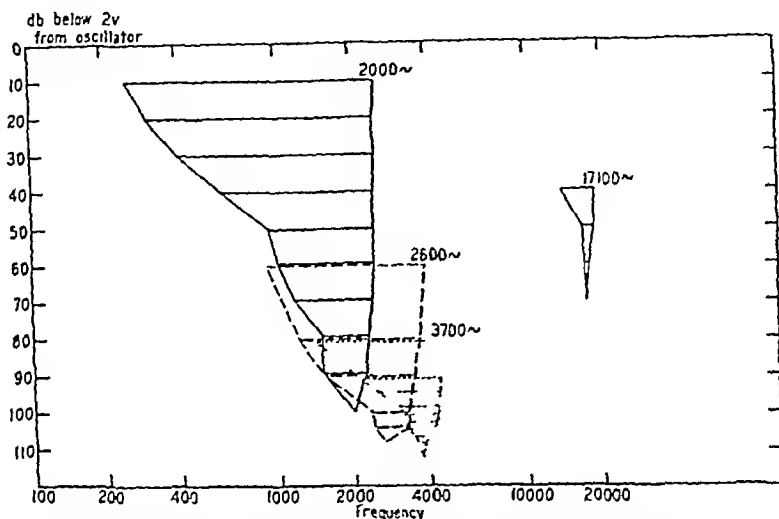


FIG 6 Response areas for 4 different fibers. The 3 at the left of the figure are from the same animal. The 2000 c.p.s. response area shown here is the same one pictured in Fig 5.

window, yet nerve fibers excited by greatly attenuated sounds of frequencies of 25 kc or higher are found with relative ease. It is also important that although over the 1 to 10 kc range the minimal intensity for some fibers lies near the threshold of the aural microphonic, other fibers may require for excitation much reduced, or much increased, intensity. This indicates that there are many nerve fibers which respond to a given frequency, and that they are called in successively as the sound intensity is raised.

The range of sound frequencies capable of exciting a fiber becomes more extensive as the intensity level is raised. This fact is shown in Fig 5. At each value given on the ordinate (intensity level), those frequencies covered by the solid horizontal line were able to excite. The sharp tuning noted at the minimal intensity obviously disappears as intensity level is raised. At 90 db above its minimal intensity, this particular fiber is excited by all frequencies between 250 c.p.s. and 2500 c.p.s., representing, respectively, 3 octaves below, and only about  $\frac{1}{2}$  octave above the characteristic frequency. These

partially adapted auditory fiber respond to increase in stimulus intensity by an increase in rate of discharge. About 400 discharges per second is the maximum rate, and a rapidly adapting fiber attains this rate when intensity is about 30 db above its minimal.

Exceptions to the above generalization are occasionally found. One fiber (1150 c p s, -80 db) discharged at a constant, slow rate (about 20

per sec) regardless of stimulus intensity. Others, like that shown in Fig 9 (10,000 c p s, -120 db) displayed marked reluctance to increase rate and never approached a maximum of 300 discharges per second. Results of this sort constitute a small percentage of the data and may eventually require special consideration and classification.

The behavior of the completely adapted single fiber to change in intensity is similar to what has just been described for unadapted or partially adapted fibers. Figure 10 shows segments from the continuous record of the response of a 2600 cycle fiber (the same one shown in Fig 7) to a 2600 cycle tone, between each segment of the record, the intensity was raised 2 db. Figure 10 and the accompanying graph demonstrate that increase in intensity is followed by a higher rate of nerve discharge in an adapted auditory fiber. The maximum rate achieved, about 200 discharges per second, lies well below the unadapted maximum of 450 spikes per second.

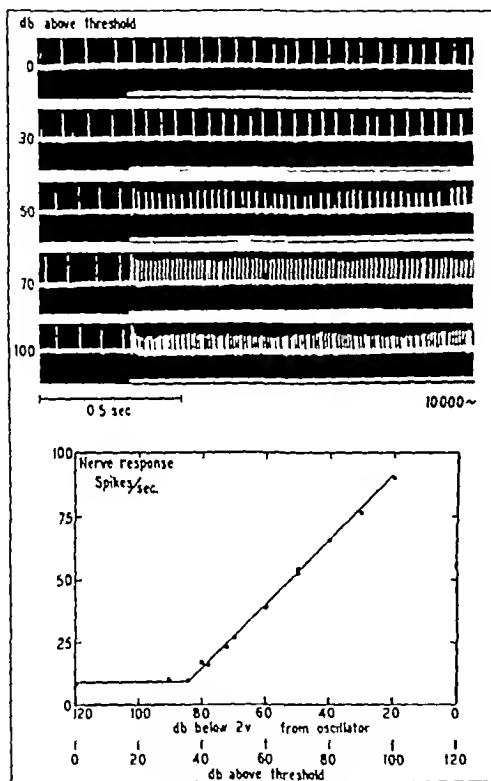


FIG 9 Frequency of nerve discharge as a function of sound intensity. This atypical fiber (10,000 c p s, -120 db) not only had an extremely low minimal intensity but also reacted toward intensity increase by a linear increase in rate of discharge.

Some adapted fibers do not behave in precisely the manner just described. One fiber, subjected to the procedure used for obtaining the data summarized in Fig 10, maintained its equilibrated discharge rate of 50 to 60 responses per second in spite of an intensity increase of 50 db. No immediate explanation for this phenomenon is apparent.

*E Iso-intensity contours for a single fiber* The rate of discharge of a single auditory fiber was shown to depend, at constant sound frequency, upon intensity of stimulus. The question now arises as to how the fiber behaves when intensity is kept constant but frequency is changed.

describe the very considerable part played by sound intensity in determining nerve discharge rate

"Minimal intensity" has been defined as that intensity level which just causes discharge, or, if the fiber is spontaneously active, where a just perceptible increase over the spontaneous rate occurs. When stimulus intensity is raised above the minimal the fiber responds by discharging more rapidly. Figure 7 illustrates this fact, in Fig. 8 data from three different fibers are

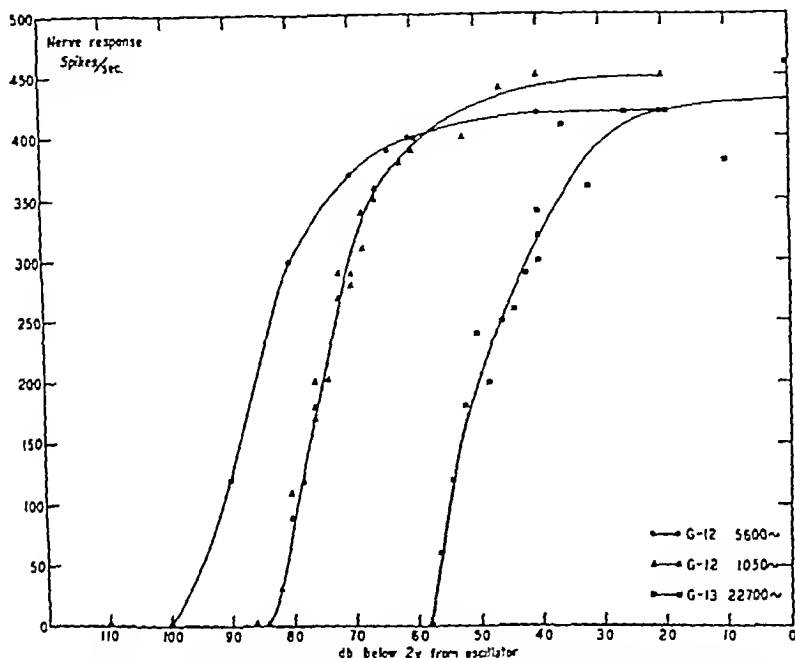


FIG. 8 Graph showing increase of nerve discharge rate with increase of sound intensity for 3 different fibers. Points determined from records like those in Fig. 7 by counting spikes occurring during first 0.1 sec. and multiplying by 10.

plotted. Frequency of nerve discharge (spikes per second) clearly varies with stimulus intensity for auditory fibers as for other sensory fibers. For most auditory fibers the maximum discharge rate is attained at sound intensities about 30 db above the minimal intensity.

The values of discharge rate plotted in Fig. 8 were calculated by multiplying by ten the spikes appearing in the first one-tenth second after onset of the tone. Reading the records in this way gives data on the maximum rate of discharge—on fibers undergoing rapid adaptation. When, instead of this, the total number of nerve discharges in the first second are counted and plotted, the resulting curves are like Fig. 8, except that lower values appear at each intensity and a maximum of 300 to 350 discharges per second is obtained with highest intensities. Typically, therefore, both the unadapted and the

given fiber. Such contours show clearly that frequency of nerve discharge is not solely determined by stimulus frequency. Instead, nerve-discharge rate is a function of both the frequency and the intensity of the stimulus. To be sure, the sound frequency which first excites (in this case 7000 c p s) is the most effective at any intensity, yet as intensity level is raised, activity is caused by frequencies at some distance from (and particularly those below) the characteristic frequency.

*F Phase relationship between nerve discharge and aural microphonic.* Volley theories, as formulated by Troland (17, p. 44) and Wever and Bray (19), postulate a close relationship between sound frequency and nerve-discharge rate. The frequency of nerve discharge according to that theory should be either equal to, or some sub-multiple of, the sound frequency. A study of

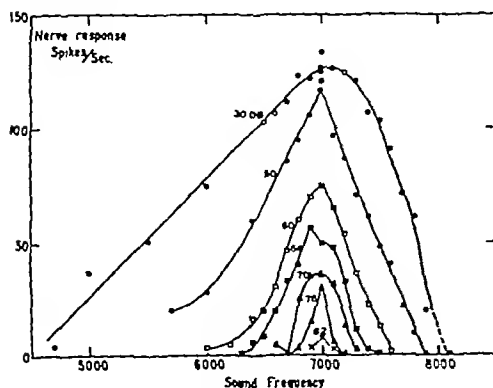


FIG. 11. Iso-intensity contours for a single fiber (7000 c p s, -84 db). The way in which frequency of nerve discharge varies with sound frequency is plotted here. The number on each contour line indicates the intensity level at which the determinations were made. This figure shows that as sound intensity level increases (numbers on contour lines get smaller), 1) the fiber is excited by frequencies which lie farther away, and 2) any frequency capable of exciting the fiber elicits more discharges. Frequency of nerve response is determined by both the frequency and the intensity of the sound stimulus.

Fig. 8, however, makes it appear that any frequency of nerve discharge (up to about 450 per sec) may accompany any sound frequency, while a cursory glance at records such as those in Fig. 1 and 3 may lead to the conclusion that the nerve discharges in a haphazard and erratic manner. Such behavior if demonstrated, would be inconsistent with a volley theory, and we were therefore interested in designing an experiment to show whether the nerve impulse is synchronized with some phase of the sound wave.

To test whether the nerve discharge occurs at a particular and specific point in the sound wave cycle, both nerve response and aural microphonic were photographed simultaneously. This was made possible since under certain conditions the potential led off by the microelectrode included a significant component from the cochlea (aural microphonic) in addition to the nerve response. Although this meant introduction of an undesirable artifact into most of the records, it supplied exactly the conditions for relating nerve response to the phase of the sound stimulus.

In Fig. 12A responses of a single fiber are seen to clump closely about one half or less of each aural microphonic cycle. The aural microphonic in this record may be taken to represent the sound wave, and since the nerve spike shows a consistent phase relationship to the aural microphonic, this indi-

This question was attacked experimentally as follows. Once the response area pictured in Fig 5 was determined for a given fiber, records of nerve activity were photographed at appropriate frequencies for each intensity

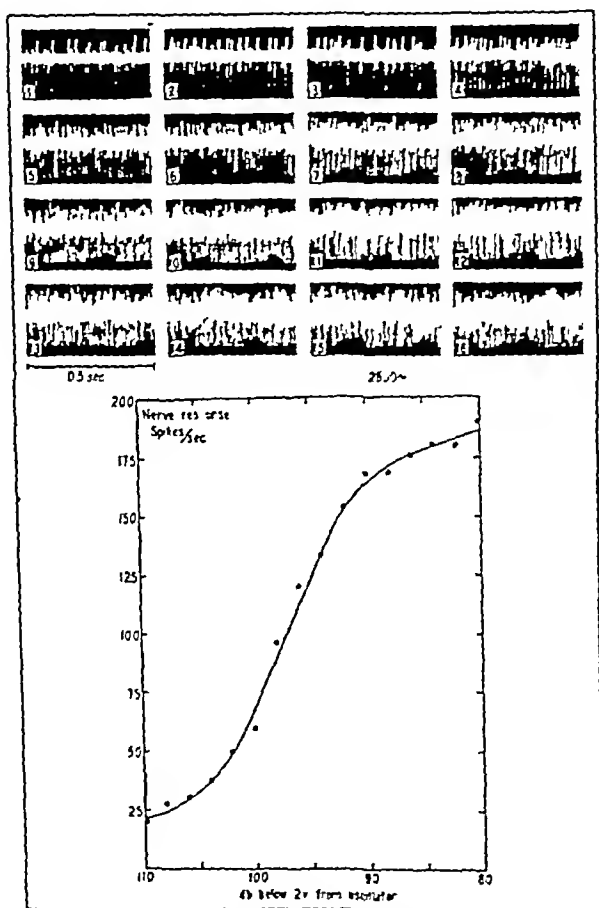


FIG 10 Frequency of nerve discharge as a function of sound intensity for an adapted fiber (2600 c.p.s., -110 db). A 2600 c.p.s. tone was delivered continuously to the animal while the intensity was raised in 2 db steps. The successively numbered segments of record in the figure represent the adapted response of the fiber to 2600 c.p.s. as intensity successively changes from -110 db to -80 db in 2 db steps. Points on the graph were established by counting the number of spikes appearing in 1 sec.

level and the spikes appearing in the first second counted. Figure 11 shows the results for one such experiment. Each contour line relates rate of nerve discharge and sound frequencies at a fixed intensity level. The whole family of curves so obtained may be termed the "iso-intensity contours" for the

activity in a single auditory unit and the familiar threshold of hearing curve. Both are defined in terms of the frequency and the intensity of the tone striking the ear and they both pass through a minimum. The principal difference lies in the relative narrowness of the frequency range for the single fiber.

The absolute sensitivity of auditory units apparently varies over a wide range (Fig. 4). The more sensitive units are just excited, at their characteristic frequencies, by intensities which must lie close to the absolute threshold of hearing. The less sensitive units, however, require for excitation intensities which must be "loud" to the cat.

Regardless of whether the unit is sensitive or not, however, an increase in intensity of 2 db over the minimal intensity causes it to discharge, on the average, about 40 additional times each second (see Fig. 8). This is a very noticeable change in the response of the unit. It amounts to about 10 per cent of the maximum possible change.

It is interesting to compare this differential intensity sensitivity of the isolated auditory unit with that of the ear as a whole. The difference in intensity is usually given as about 3 db between 1000 and 4000 cycles at 5 db above threshold for human subjects (16, p. 138). The 3 db increase of intensity required to elicit a sensation of increased loudness (human) will cause about 50 additional discharges per second to arise in one auditory fiber (cat).

*2 Spontaneous discharge, silent period and after-discharge.* Spontaneous activity in sensory nerves appears to be a fairly common phenomenon. Thus many visual (8), olfactory (3), gustatory (15), lateral line (9), and now auditory afferents are known

to discharge in the absence of any apparent stimulus. On the other hand, vestibular (12), touch (1, 5), visceral (10), vibratory (14) and striated muscle (13) afferents seem to display little if any spontaneous activity. Spontaneous activity appears to occur only when the nerve is in continuity with an active end organ. Lateral-line afferents cease discharging if cut peripheral to the recording electrodes (9). Adrian and Ludwig (3) showed that whereas spontaneous discharge in the nerve was reduced by anesthetizing

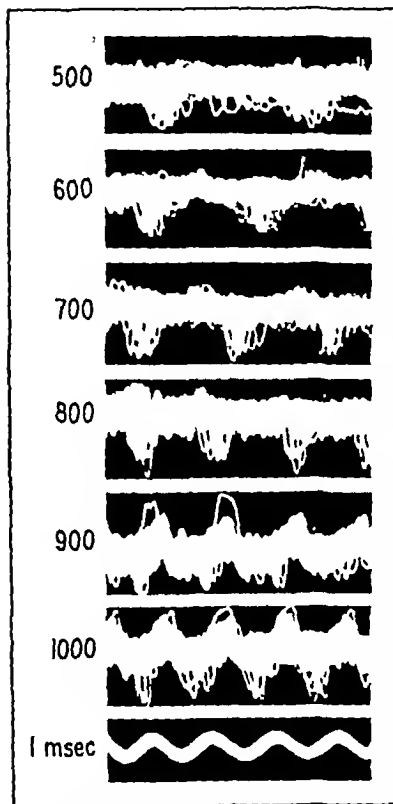
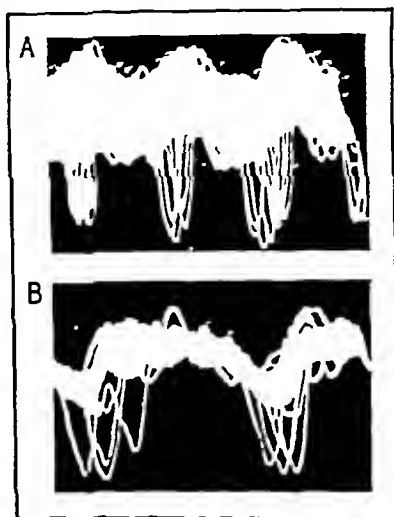


Fig. 13 Simultaneous recording of aural microphonic and single fiber response at different frequencies. This fiber (650 cps, -52 db) discharges in phase with the microphonic at frequencies between 500 and 1000 cps.

cates a similar phase relationship to the sound wave. The hundreds of sound wave cycles (measured, as just indicated, in terms of microphonic cycles) recorded in the sweeps in Fig. 12A gave rise to only tens of nerve discharges, yet it is perfectly clear that nerve impulses, when they do arise, consistently occur at a specific and particular portion of the sound wave cycle. Figure 12B shows the similar behavior of another fiber. Figure 13 illustrates the same point, and supplies the additional information that the nerve discharge is in synchronism with any sound wave of sufficient intensity to stimulate. These results both confirm and extend to volley hypothesis.

It will be noted that there is some variability in the spot on the aural

FIG. 12 Simultaneous recording of aural microphonic and single fiber response. Each record is the photograph of many sweeps on the cathode ray tube face. The responses of the nerve are the sharp downward deflections, aural microphonic is the undulating heavy line. A, 1050 c.p.s., -16 db delivered to the ear (minimal response from the fiber at 1050 c.p.s., -32 db). B, 550 c.p.s., -24 db delivered to the ear (minimal response at 550 c.p.s., -40 db). Records photographically intensified but not retouched.



microphonic cycle where the nerve impulse arises. The maximum variability amounts to about 0.25 msec. in Fig. 12A. If it be assumed that a similar variability occurs at other frequencies, it would be expected that the auditory nerve as a whole could not discharge synchronously above about 4000 c.p.s. This is the value arrived at experimentally by Wever and Bray (18) and by Derbyshire and Davis (4).

## DISCUSSION

### *A. The behavior of auditory nerve fibers*

*1. Sensitivity.* The threshold for excitation of a given auditory-nerve fiber is given by the series of points which marks the boundary of the response area (Fig. 5 and 6). Each point on this contour designates a tone which is just adequate for exciting the fiber. The point at which this contour passes through a minimum of intensity gives the "characteristic frequency" and "minimal intensity."

There is a resemblance between the contour showing the threshold for



at most for 10 per cent of the total voltage drop undergone during equilibration, while rate-adaptation probably accounts for the remainder. Ordinarily the rate drops to 25 per cent of the maximum within a second after onset of stimulation, and some fibers, especially those not maximally excited by the tone, may adapt to extinction. Although it is difficult to assess the importance of rate-adaptation in rapid equilibration with any exactness, it seems clear that it may decrease the potential output of the auditory nerve to 25 per cent of its maximum within a second or so after the sound goes on.

*4 Maximum rate of discharge* The maximum rate of nerve discharge appears to be determined not by the ability of the fiber to respond but by the capacity of the end organ to discharge. During the first 0.05 sec. after onset of stimulation, a series of impulses at a maximum rate not exceeding 500 per sec. may arise, yet one impulse can follow another at rates up to 900 or 1000 per sec. This seems to show that the nerve is only occasionally called upon to discharge at its highest rate.

The bottom sweep in Fig. 1C was reproduced to show the shortest interval between two impulses noted in some 500 high-speed records. The interval is about 1 msec. Intervals shorter than this—indicating rates higher than 1000 per second—have not been observed in hundreds of slow-speed records either, there is no justification for assuming, therefore, that auditory nerves display unusually brief refractory period phenomena.

### *B Single fiber responses and hearing*

*1 General considerations* It is necessary to establish one point regarding the distribution of the peripheral endings of the auditory nerve in order to apply the data from single fibers to a specific theory of action of the mammalian cochlea in hearing. The question is simply this: are the endings of each fiber in contact with few or with many hair-cells? Lorente de N6 (11) discussed this problem and described five types of fibers, of which "radial" fibers end on a few neighboring internal hair-cells, while "spiral external" fibers end on hair-cells over a third of a turn or longer. The distributions of only two out of five fiber-types are known, and thus the question of how many hair-cells are capable of exciting a given nerve fiber receives only a partial answer from the excellent work of the anatomists.

Pending clarification of this problem, it will be assumed that each nerve fiber is in contact with a "small number" of hair-cells. From this assumption it follows that response in a single auditory fiber means excitation in a particular and restricted region of the cochlea. It will be noted that distortion of hair-cells resulting from movement of the basilar membrane is taken for granted as a prerequisite for nerve excitation. This train of events appears to be generally accepted as a fundamental part of the mechanism of hearing and needs no further discussion.

*2 Pitch discrimination near threshold* In Fig. 4 the frequency and intensity at which minimal response was obtained from some 40 separate auditory fibers are plotted. At minimal intensity, each fiber responds to

the end organ (olfactory bulb in fish), it could be abolished only by section of the nerve between the end organ and the recording electrodes. It is difficult to say exactly what constituted the olfactory stimulus in the above case, and indeed to determine whether there actually was one, the same difficulties arise in the case of the eye, tongue and ear. Two alternatives are apparent either it is not possible completely to eliminate stimulus energy under even the best experimental conditions (*i.e.*, there is no such thing as a "stimulus-vacuum"), or a certain number of end-organ elements, always delicately poised at the brink of discharging, occasionally do so even though no stimulation of the sort usually considered adequate is presented. As an example of the latter alternative, slight mechanical movements induced by blood flow might be responsible for all spontaneous discharge. If this were true, it would appear that the cells of a given end organ, irritable to an extraordinary degree to one type of energy, nevertheless can be discharged by other types of stimuli.

In the case of the ear, spontaneous nerve activity may be due to discharge of the end organ stimulated by the many sounds which must constantly bombard the ear. Sounds attending respiration, heart beat and flow of blood through cochlear structures as well as those external noises resulting from inability to soundproof the experimental room completely may all constitute adequate stimuli for the sensitive end-organ structures.

The silent period is that fraction of a second following cessation of stimulation during which no spontaneous activity is detectable. The silent period was observed in most fibers isolated and seemed to be particularly marked after stimulation at high intensity levels, no attempt was made to study this phenomenon in any systematic way. A marked increase in spontaneous activity followed the silent period in certain cases where stimulation was very intense (Fig. 1E). Because of analogies to behavior of other end organs, this may be termed an after-discharge. It deserves further study since it may be correlated with the tinnitus which many persons experience after exposure to loud sounds.

**3 Adaptation.** Like certain visual, muscle spindle and pressure afferents, auditory nerves respond to a continued stimulus of constant intensity by a burst of impulses which gradually declines in rate. In this respect they clearly resemble the pressure sense organs from which they are embryologically derived.

The final rate of discharge maintained by an auditory receptor adapted with tones at ordinary intensity levels is 100 to 200 discharges per second, and the steady state is arrived at within a second or two. No further significant change occurs even though activity is recorded for as long as 30 seconds. The so-called "slow equilibration" discussed by Derbyshire and Davis (4) was not investigated.

The "rapid equilibration" of Derbyshire and Davis appears to result from a combination of rate- and amplitude-adaptation occurring in the fibers excited by the stimulus. Adaptation in amplitude of response may account

allow direct exploration of the total area a given tone involves. Instead, it identifies only those tones which affect the area isolated. The evidence is nevertheless unequivocal for a gradual and systematic extension of the pattern of vibration of the basilar membrane as intensity is raised.

Here, then, is a second phenomenon common to all tones having the same pitch. They all cover the same minimum area, and the louder ones, while extending that minimum area in all directions, do so according to a series of regular rules. So long as the site of maximum stimulation remains fixed and new increments of area around the edges of that previously in vibration are added in a systematic way, the pitch stays constant.

It should be apparent that the discussion in this section very closely resembles the space-time pattern theory formulated by Fletcher (6). Similarities and differences will receive more complete attention in a later paper, in which, also, the pattern of vibration of the basilar membrane as derived from response-area data will be discussed.

#### SUMMARY

The activity in single fibers of the auditory nerve in cats has been studied with the aid of microelectrodes. The fibers were excited by delivering acoustic stimulation to the ear. Each auditory-nerve fiber responds only to a narrow band of sound frequencies when the sound intensity is just sufficient to excite it at all. Fibers were found which were specifically sensitive to narrow bands of frequencies in the frequency range between 420 c p s and 25,000 c p s (Fig 4).

The auditory fiber exhibits no unusually brief refractory phenomena. It may discharge spontaneously in the absence of any apparent sound stimulus. After a period of marked activity during sound stimulation, the spontaneous activity may be temporarily depressed (silent period), then accelerated (after-discharge) (Fig 1).

The auditory fiber typically responds to a continuous adequate sound stimulus by a train of impulses initially high, but gradually declining in rate (Fig 3). Within a few tenths of a second after the tone goes on this rate-adaptation is complete and the amplitude of the action potentials is somewhat diminished as well. It is concluded that auditory fibers behave in every important respect like other sensory fibers.

At constant frequency an increase in sound intensity causes an increase in rate of discharge by the single fiber (Fig 8). Most fibers reach a maximum of 450 discharges per second after an intensity increase of about 30 db.

The frequency band capable of exciting a given fiber increases markedly as the intensity level is raised (Fig 5). At levels about 100 db above threshold tones as far away as 3 octaves below and  $\frac{1}{2}$  octave above may be adequate.

The auditory-nerve fiber discharges in synchronism with a definite part of the stimulating sound-wave cycle (Fig 12).

The results are held to support a place theory of hearing according to

only a narrow band of frequencies. All other frequencies are ineffective. This clearly indicates that each pure tone singles out and causes to move one particular and restricted region of the basilar membrane. For perception of pitch at threshold, then, the data from these experiments confirms a place theory of hearing, a theory according to which different regions along the length of the basilar membrane are excited by different sound frequencies.

*3 Loudness* The principal psychological correlate of the intensity of a tone is its loudness. Our experiments show that an increase in sound intensity results in an increase in number of nerve impulses ascending the auditory nerve.

Increase in intensity of the sound stimulus excites more nerve activity in two ways. First, each active fiber discharges at a higher rate (see Fig. 8). Second, a larger number of fibers are made active. This is shown by Fig. 5 and 6 (response areas), where a given spot on the basilar membrane—fixed by the single fiber being studied—is excited by sound frequencies which lie farther and farther removed as the intensity level is raised. These response areas, demonstrated experimentally, can only be interpreted as supporting the familiar concept of spread of excitation along the basilar membrane as a basic explanation for loudness perception.

*4 Pitch perception at high intensities* For man at least, a 2000 cycle tone near threshold has just about the same pitch as a 2000 cycle tone at a sensation level of 100 db. Our experiments show the involvement of a large basilar membrane area, with many nerve fibers excited, when the tone is loud, as opposed to a small area with few fibers excited at or near threshold intensity (Fig. 5, 6, and 11).

However, the ability of the frequency which first excites to continue, as intensity level rises, as the most effective stimulus is a consistent finding. Thus, in Fig. 11 where the iso-intensity contours for a 7000 cycle fiber are plotted, each 7000 cycle tone calls out more nerve activity at a given intensity level than any other stimulating frequency. This particular 7000 cycle fiber was always most readily excited by a 7000 cycle tone at any intensity level up to 50 db above its minimal intensity. At intensities above this, adjacent frequencies begin to cause maximal discharge, and consequently maximal discharge from this fiber can no longer serve as a clue for pitch. However, it should be recalled that a number of fibers have the same characteristic frequency but widely different minimal intensities (See Fig. 4 at 7000 cycles). The characteristic frequency is the most effective stimulus, therefore, to that intensity where all fibers "tuned" to that frequency have not yet been forced to respond at their maximum rate. This, in effect, is a restatement of Gray's theory of maximum stimulation.

In addition to causing maximum excitation at one spot on the basilar membrane, increasingly more intense tones at a given frequency appear to enlarge the area involved in a regular and systematic manner. This is shown both by the response-area evidence already discussed, and by the iso-intensity contours. It must be clear that the single fiber technique does not



which pitch is a function of where, and loudness a function of how much of, the basilar membrane is disturbed

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tion of the boundaries of the responsive cortex. Further explorations were made in similar fashion over wide cortical areas bordering the acoustically responsive area so defined.

The primary responsive area was next strychninized by applying to it a small piece of blotting paper moistened in a 1 per cent aqueous solution of strychnine sulfate. The previous explorations were repeated and other procedures were carried out as detailed below.

### RESULTS AND DISCUSSION

Ten cats were used. In all cases after strychninization of the primary responsive area, responses resembling the diphasic primary projection response occurred in a second, hitherto silent, region of the posterior ectosylvian gyrus. (See Fig 1 and 2.) Usually there was a non-responsive area

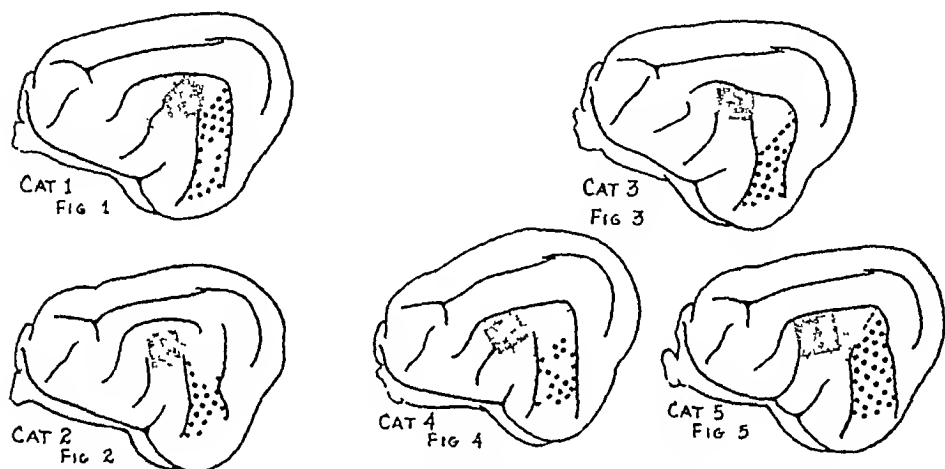


FIG 1 and 2 Lightly stippled area on middle ecto-sylvian gyrus shows primary projection area, heavily stippled portion within primary projection area indicates extent of strychninized cortex. Secondary responsive area is shown by heavy dots.

FIG 3, 4, 5 Same symbols as Fig 1 and 2. Broken line indicates position of incision made in cortex.

separating it from the primary area. In all cases, whether or not the two areas were contiguous, maximal secondary potentials were obtained from the upper part of the lower half of the posterior ectosylvian gyrus, and the magnitude of response diminished from that center in both superior and inferior directions. In no case was there a secondary responsive area anywhere except on the posterior ectosylvian gyrus.

The potentials from the secondary area could always be diminished by making a shallow (2 mm) cut in the cortex across the posterior ectosylvian gyrus at the line or strip of demarcation between primary and secondary areas. (See Fig 3, 4, and 5.) They could sometimes be entirely abolished by deepening the incision to 4 mm. In three cats, after determining the extent of the secondary responsive area, the primary area was decorticated by suction pipette. In each case, after such decortication, no vestige of response remained in the still intact posterior ectosylvian cortex.

# A SECONDARY ACOUSTIC AREA IN THE CEREBRAL CORTEX OF THE CAT

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PRESENT knowledge of the type of cortical association area known as para-receptive cortex (*e g* peristriate and parastriate areas) is based principally on cytoarchitectonic studies purporting to demonstrate that such regions of specific histological structure are related to associative functions. In addition, there have been several reports of the distribution of fibers from primary receptive zones to surrounding cortex, based on Marchi studies following experimental lesions in the primary areas. There is a division of opinion arising from these studies concerning the distance traversed by and pattern of distribution of such association fibers. In some instances bundles of fibers have been described which run for long distances to terminate in remote parts of the cortex. In others, there seems to be reason to believe that the frequently described long association bundles are improbable and that transcortical conduction is accomplished by fibers running only short distances. Presumably these are arranged in some sort of chain or network in such a way that impulses taking origin in a given locus may ultimately reach remote cortical loci. This hypothesis is difficult to correlate with the observation of several investigators that stimulation of a cortical locus may result in an electrical response in some other specific cortical locus, both loci being surrounded by great areas of non-responsive cortex.

The present communication is a report of experiments using the strychnine method on the temporal cortex of the cat, they grew out of and were incidental to the oscillographic mapping of the acoustic projection area. While the idea that strychnine may act upon cortical (or other) synapses in such a way as to facilitate synaptic transmission is not new, the application of this principal to a study of transcortical conduction pathways has been rather limited.

## PROCEDURE

One or both hemispheres of the cat were exposed under deep Nembutal anesthesia. The areas of cortex showing responses to click stimulation were then mapped by the method described by Ades (1). Cortical response to sharp mechanical clicks delivered one foot (30 cm) from the cat's ear was measured by means of a single-phase, capacity-coupled amplifier recording on a cathode-ray oscillograph. The active electrode was a saline-moistened thread drawn through a hypodermic needle and anchored to the skull by a holder permitting movement in any direction; the animal was grounded through a clip-lead attached to skin or muscle. The electrode, in light contact with the pia mater, was moved systematically in 2 mm steps over an area sufficiently large to permit exact defini-

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definitely is acoustic in its connections and function (2) In the light of the recent studies cited above it is clear that the termination of the geniculotemporal radiation in the cat has as its lowest possible inferior limit, the superior end of the pseudosylvian sulcus, more probably the limit is 2-3 mm above the end of the sulcus The situation in the cat is complicated because

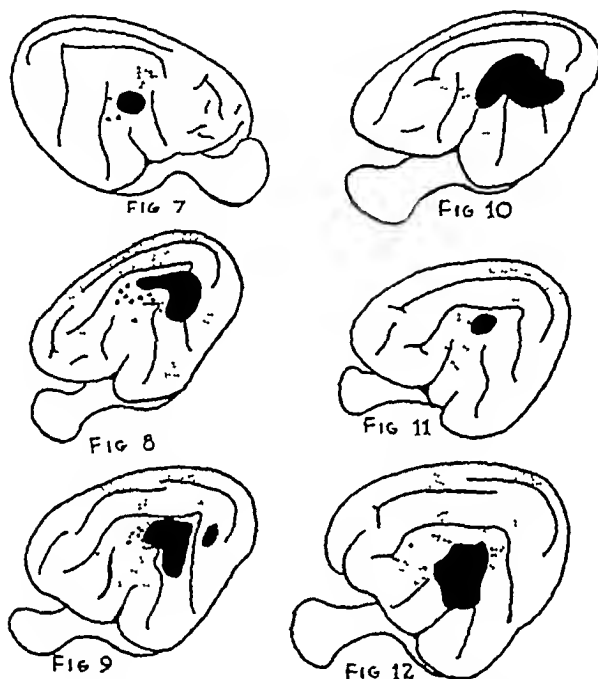


FIG 7-12 After Mettler Solid black marks lesions Stippling indicates points of termination of degenerated fibers from area of lesion, proportionate number of fibers is shown by relative size of dots, heavy dots denoting strong connections

- FIG 7 Mettler's cat 513, Fig 13  
 FIG 8 Mettler's cat 516, Fig 11  
 FIG 9 Mettler's cat 517, Fig 11  
 FIG 10 Mettler's cat 514, Fig 17  
 FIG 11 Mettler's cat 505, Fig 21  
 FIG 12 Mettler's cat 507, Fig 9

of the complete lack of a middle ectosylvian sulcus, rendering problematical the exact line of demarcation between pseudosylvian and middle ectosylvian cortex If compared with the canine temporal region, it seems most likely that the lower limit of the geniculotemporal projection area of the cat corresponds to the middle ectosylvian sulcus of the dog (in the dog, the acoustically responsive area is confined to the cortex bounded inferiorly by the middle ectosylvian sulcus)

Since Campbell's map is apparently impossible of confirmation even by

The most obvious explanation of an area such as this, is that it constitutes a parareceptive zone, or acoustic association area. From the evidence adduced, one would expect to find strong fiber connections from middle ectosylvian cortex (primary acoustic area) to the posterior ectosylsylvian region of secondary response. One might equally well expect that the two areas would differ in their histological features. As a matter of fact, neither condition obtains in the light of available anatomical evidence.

Campbell (4), on the basis of cytoarchitectonic studies, divided the temporal cortex of the cat into two parts which he called ectosylvian A and ectosylvian B (See Fig 6). Obviously, neither area corresponds to either of the areas described in the present study (*i.e.* primary projection area or pos-

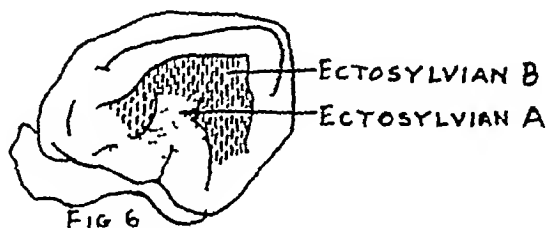


FIG 6 Cat temporal areas according to Campbell

terior ectosylvian secondary area) Mettler (6), in the course of an investigation of the cat's temporal region by the Marchi method, describes six cases in which his lesions involve considerable portions of the primary acoustic area as defined independently by Kornmuller (5), Bremer and Dow (3), and Ades (1) (See Fig 7-12). In four of the six cases, degenerated fibers to the area which has been designated as secondary acoustic are conspicuously absent, in the other two cases, such fibers are sparse, and, judging from Mettler's description, among the least striking of the short association connections from the middle ectosylvian cortex. In short, the areas delineated by both Campbell and Mettler fail to coincide with the experimental findings described above.

At this point it may be pertinent to note that the only point of agreement which can be found in the feline temporal area is on the location of the primary projection area. By the use of no less than four different methods, Kornmuller (5), Bremer and Dow (3), Woollard and Harpman (7), and Ades (1) define almost precisely the same primary projection area. There is, thus, a serious disagreement between the cortical map common to this group of workers and the map of Campbell which Mettler apparently finds himself able to confirm.

It is difficult to consider seriously any map which, like Campbell's, places acoustic function in the carnivore pseudosylvian cortex. That area corresponds to the insular cortex of the primate which is certainly not acoustic. The carnivore ectosylvian region, on the other hand, is presumably homologous with the superior temporal cortex of the primate, and the latter most



his own method (3), it is no longer acceptable. If it be rejected, it is possible to reopen the problem of interrelations within the feline temporal cortex. Therefore, in addition to the primary projection area occupying the middle ectosylvian gyrus, the experiments reported here indicate the presence of a secondary acoustic area on the posterior ectosylvian gyrus.

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wardly along the floor. In general, however, voluntary movements of the animal at this time were even slower than normal for the sloth. Further section of the whole cortex was made usually within 2 hours, to the depth of several millimeters lower than the first incision.

The completely decorticate two-toed animal showed itself vigorously aggressive (for the sloth) on merely slight provocation. The flexion striking movement of the fore limbs was fairly quick and powerful, although erratic, teeth were bared, and clawing, biting and grinding actions ensued, snarling and hissing were common features, and continued many minutes after a suspected adversary had retreated. Salivation occurred in a few cases. Sweating was not observed on the foot-pads or any part of the body at any time. It may be mentioned, however, that at least vestigial sweat glands are said to be present in the sloth (2). The quasi-emotional expressions were usually lower in force value than affective reactions found in normal animals, but showed longer, irregular and unbridled continuance.

In the case of the three-toed sloth, pseudoaffective responses were somewhat similar but much less vigorous, and striking movements were usually

*Table 1 Decorticate and Decerebrate Reactions in Sloths*

Animal No	Species	Days Observed after Operation	Level of Brain Section	Rectal Temp °C	Pseud-affectivity	Dominant Rigidity	
						Flex-ion	Extension
1	2-Toed (Chol hoff)	2	Ant Coll	30.9	+++	+++	0
2	" "	4	Low	32.8-30.5	+++	+++	0
3	" "	7	Ant Coll	34.2-34.8	+++	+++	0
4	" "	6	Low	29.3-32.2	++	+	+++
5	" "	4	Post Coll	33.5-33.9	+	+	+++
6	" "	3	Post Coll	34.9-35.5	+	+++	+
7	" "	2	Ant Coll	34.0	+	+++	+
8	" "	6	Ant-Post C		+	+	+
1	3-Toed (Brad g g)	1	Low		0	+++	0
2	" " "	2	Low	32.9	+	+	+++
3	" " "	2	High	33.7	+	+	+++
4	" " "	6	Low		0	+	0

absent. The expressions were really in nearer agreement with the normal behavior of this most torpid of all mammalian types. The results of these experiments are given in summary in Table 1.

There was persistence of pseudoaffectivity in nearly all animals even after several brain sections had been carried out, and the immediate consequent shock had disappeared. The condition was apparent to some extent after transections in the vicinity of the anterior colliculi. In the case of low-level or decerebrate preparations, however, such periods of (induced) activity ap-

# THE PSEUDAAFFECTIVE STATE AND DECEREBRATE RIGIDITY IN THE SLOTH\*

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THE EXCEEDINGLY low plane of activity on which the sloth lives offers a stimulating challenge to naturalists and experimental workers alike. In studying various functions of this animal recently in its native habitat (2), it seemed desirable to inquire into some phases of cerebral influence on muscular activity. Different ways of speeding up or deslothing the sloth, including excitation of the central nervous system, have already been reported (5). Such stimulation of this ancient mammal, however, does not bring it anywhere near par in a muscular sense with modern, active forms.

The commoner sloths, the two-toed *Cholepus hoffmanni*, and the extremely slow three-toed *Bradypus griseus griseus*, were studied in the present experiments. All observations refer commonly to both didactyl and tri-dactyl forms, unless otherwise noted.

## METHODS

Under light preliminary ether anesthesia, a few square centimeters of the scalp in the mid-region were deflected, a 2-cm trephine opening made in the cranium, and the rest of the bony vault then removed with rongeur forceps to expose the upper brain surface in its coverings. In earlier work some attempts were made to localize cortical motor areas by electrical stimulation, after cutting and drawing aside the dura mater, these efforts were much handicapped, however, under tropical conditions. More specific inquiry was then made into the possible occurrence of the pseudoaffective state in both sloth forms. Cerebral transections were performed with a semi-blunt dissector, in order to reduce bleeding to a minimum. Since the blood pressure in the peripheral tissues of sloths is low (2), ligation of carotid vessels was not necessary. At autopsy the remaining brain tissues were removed and examined after fixation in formalin.

## RESULTS

The first sections of the cerebral cortex extended over the frontal and parietal regions, and were carried to the depth of a few millimeters only. Shock was observed to supervene in all cases, the atonic, unresponsive condition was usually profound for about 5 minutes, and gradually gave way in the next 8 or 10 minutes to a nearly normal responsive state. In some cases, however, shock was in evidence for 20-30 minutes.

Such partially decorticate sloths became spontaneously active within an hour after operation, and began climbing about the cage or crawling awk-

\* The physiological phases of these studies were carried out at the Gorgas Memorial Laboratory, Rep. Panama, under the terms of a John Simon Guggenheim Memorial Fellowship held by the senior author during 1937-39. The thanks of the authors are gratefully extended to Dr. Herbert C. Clark of this laboratory, for his unfailing courtesies during the investigations.

Some features of the posture in the decerebrate preparation are shown in the adjoining illustrations (Fig 1) Decerebrate sloths survived several days—in some cases 6 or 7—after cerebral section had been carried out No attempt was made to maintain asepsis, and apparently infections were responsible in a few instances for early exitus

Rectal temperatures were recorded throughout, and it will be seen from Table 1 that no marked change followed decerebration Earlier work in this laboratory shows that the rectal temperature of sloths is normally several degrees below that of higher mammals (3, 4, 5), and those now recorded are in agreement The average readings in our experiments were

normal two-toed sloth,	34.4°	three-toed, 33.0°
decerebrate two-toed sloth,	33.0°	three-toed, 33.3°

Pulse and respiratory rates were not significantly different from those of the normal, unoperated sloth

### DISCUSSION

The sum total of the decorticate reactions in the sloth constituted a fairly complete parallel to the pseudoaffective or quasi-emotional state first observed by Woodworth and Sherrington (9) Similar in character, pseudoaffectivity in this ancient and primitive form was less evident in degree only compared to that described in higher mammals by Cannon and Britton (6)

Decerebrate rigidity of the extensor type occurred frequently in these experiments, but not as often as the flexor type, which was first described by Richter and Bartemeier (8) These earlier investigators did not observe extensor rigidity in the sloth, possibly because of failure to study their preparations over a sufficiently long period after operation In four of our surviving cases, hypertonus in extension became a notable feature as the condition of flexion regressed Bazett and Penfield (1) also found differences in the character of tonic decerebrate responses at different periods after operation Furthermore, our animals were utilized in the fresh state in the tropics, shortly after being brought in from the jungle

The extensor rigidity appeared to be typical or classical in character, not unlike that in the decerebrate cat Differences in the response of the decerebrate sloth, *i e* flexion or extension under varying conditions, may perhaps be referable to the level of brain section, but on this point our results are not clear

Langworthy (7) showed that, although the flexors in sloths may represent the anti-gravity muscles, extensor responses of the limbs on stimulation of the cortical motor areas are nevertheless predominant That the muscle mass of the commoner tardigrades represents only about 25 per cent of the body weight, compared to 40 per cent in most mammals, has already been pointed out (2) The marked hypertonicity which may be developed in the decerebrate sloth is therefore particularly striking, in view of the relative poverty of skeletal muscle in this animal

peared only occasionally, and intermittent outbursts of rigidity were frequent

In a few of the earlier experiments on decerebrate sloths, the rigidity which was first observed was wholly of the flexor type. The flexion movements were usually extreme, and the animal sometimes remained curled up in a resistant, ball-like mass for 5-15 minutes at a time. In a few instances the forelimbs only were held tightly clasped across the chest and abdominal area. In tridactyl animals, the stumpy tail was usually extended or dorsiflexed.

It was a common finding in many of these experimental animals, however, that decerebrate rigidity of the extensor type also supervened during a part of the post-operative period. In some cases, indeed, this was the dominant condition. The extensor position which was assumed was essentially



Typical extensor rigidity in decerebrate sloths. (A) Two-toed animal, *Cholepus hoffmanni*. (B) Three-toed, *Bradypus griseus griseus*. Note that feet are held in the position of slight flexion. The hind limbs of both species are not straight even in the 'fully extended' position.

similar in both didactyl and tridactyl forms, although *Cholepus* usually gave the better display, both fore and hind limbs were involved, and dorsiflexion of the head was seen when the animal was placed on its side and thus allowed freer movement. Slight flexion of the feet occurred almost invariably in correlation with extension of the limbs. In several instances extensor rigidity appeared more prominently in the latter part of the post-operative survival period, when in contrast flexion usually tended to disappear.





## SUMMARY

1 Shock which follows transection of the brain in the sloth is profound for about 5 minutes, and usually disappears in 10-15 minutes

2 Considerable activity, apparently spontaneous, is shown by sloths which have been deprived of large cortical areas, including those dominating motor reactions in higher forms

3 A pseudoaffective state, not significantly different from that observed in higher mammals, appears following removal of the upper parts of the tardigrade cortex. This condition may persist even after brain section has been made to low levels

4 Decerebrate rigidity in the sloth may be either flexor or extensor in type, the former is somewhat more frequent. Extensor rigidity is usually more evident during the latter part of the post-operative survival period, and is classical in type

5 Temperature, pulse and respiratory rates were well maintained in the decerebrate sloth

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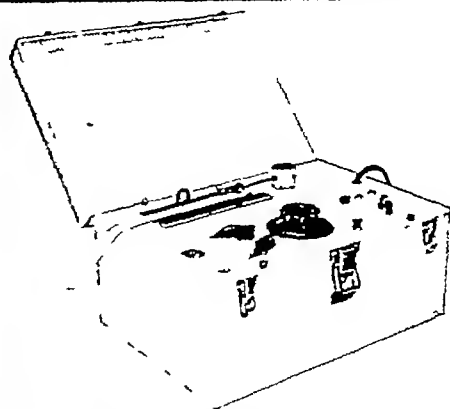
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## RESULTS

*Absence of attacks at 0° or below in North American frogs* In a number of experiments performed at New Haven with North American frogs it was possible to confirm the point already observed, namely, that attacks were not caused at temperatures above 0°C In the majority of cases there was no reaction The muscles of the isolated hind limbs remained perfectly immobile, even though the cord were exposed to the low temperature for ten, fifteen and even twenty minutes In several cases, although no attack was produced, there were fibrillary twitchings similar to those which precede ordinary attacks The twitchings were feeble and usually lasted only one to two minutes

Only once was a full attack produced, the temperature of the bath was 0.5°C, the attack tonic and clonic, long and prolonged, commenced 7.5 minutes after the beginning of the chilling This resilient form lasts from 1 to 1.5 minutes after the cord is plunged into the bath It is not easy to explain this single exception contrary to all our experience in other North American frogs The animal apparently was not abnormal

In previous experiments it was found that attacks developed without external excitation, sections of the posterior nerve roots did not prevent them External excitation, however, did have some influence upon the attacks Repeated stimulation of the foot pads during chilling of the cord might lead to an attack at a temperature several degrees above the superior limit at which attacks ordinarily occurred The reflex influence was ipsilateral In North American frogs, reflex attacks could not be demonstrated under 0° but twitching could be induced and pronounced chronic seizures did not develop

In resumé, epileptiform attacks caused by sudden chilling of the spinal cord of North American frogs can not be caused in temperatures above 0°C

*With cord exposed* Experiments with Brazilian frogs had shown that the speed of chilling exerted a considerable influence on the attack If, in place of being sudden, the chilling proceeds slowly, low temperatures may be attained without causing an attack The opening of the vertebral canal, with the cord exposed and submitted to direct action of cold, accelerates the chilling The latent period, that is to say, the time between the onset of action of the cold and the beginning of the attack was reduced to about 10 seconds, if the temperature of the bath is below 4°C Otherwise, one can cause an attack up to 12°, whereas the upper limit is 8.5° and the vertebral canal remains closed

In North American frogs it is possible to obtain a characteristic attack after exposure of the cord The operation is performed one half hour before the experiment Before making the preparation, the determination whether the animal's voluntary movements are normal is made, and after preparation, whether the reflexes are in good condition, and finally assurance that neither the cord nor its roots have been injured during the operation

Under these conditions only once was there no attack In all other ex-

# INVESTIGATION OF EPILEPTIFORM ATTACKS PRODUCED BY SUDDEN COOLING OF FROG SPINAL CORD

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IN 1933 it was found that sudden cooling of the spinal cord of Brazilian frogs with cord and posterior extremities isolated, caused an epileptiform attack. No one previously had obtained attacks of this type in frogs or in other animals. Faradic stimulation was without effect, also constant current stimulation. (2) In re-examining the question with various types of stimulating currents, it was determined that Brazilian frogs did not develop attacks with any type of electrical stimulation.

Detailed studies were accordingly made of cold seizures. One point hitherto unstudied is presented in this investigation, namely, that the attacks exhibit characteristics different in different species of frogs and they show considerable modifications in the same species transferred to a different climate. The influence of climate and other conditions in which the animals live has an influence on the characteristics of seizures. Also for the South American frogs (*Leptodactylus ocellatus*) the upper limit of temperature is higher at Rio, 8.5°C than at Buenos Aires or Montevideo, 5°C, and higher still at Pernambuco 12°C than at Rio. The form of the attack is the same at Buenos Aires and at Montevideo where climatic conditions are closely similar. It has been demonstrated also that North American frogs transported to Rio do not have attacks even at 0°, indeed one cannot even induce them by lowering the temperature several degrees below zero. The change in character of the epileptiform attacks, the differences of species and conditions of life raise interesting questions. The present study describes experiments made at Yale with North American frogs during the winter of 1942.

## TECHNIQUES

Several different procedures were followed for recording an attack by sudden cooling. In one group it was arranged to use Ringer solution so arranged as to be chilled quickly by circulating it through ice water previously described. The isolated spinal cord preparation could be plunged quickly in a cold Ringer bath with the hind extremities attached to a cord through the sciatic nerve, rested outside the bath and arranged for myographic recordings. The temperature of the bath was recorded by a simple thermometer. When it was necessary to cool the cord below 0°C it was still possible to use the bath method replacing the Ringer by liquid paraffin (petrolatum) or even by a concentrated sodium chloride. It has been established that at extremely low temperatures hypertonic solutions do not stimulate nerves. Another method consists of applying ether, or better, ethyl chloride, on the unopened vertebral column. It is necessary to place the preparation in a position so that the ether or ethyl chloride does not fall on the nerve trunks as they emerge from the cord. One can also evoke attacks by applying carbon dioxide snow on the vertebral column. These experiments have been used with "dry ice."

The experiments of control are made with preparations in which the cord has been previously at the interior of the vertebral canal. However, the application of dry ice on the dorsal surface of the spinal cord gives place to contractions of the muscles of the legs. These contractions due to chilling of the nerves through the other tissues begin much later than in the case of the attack, their duration is much more reduced, they are much less intense, not presenting a tonic phase and not showing a systematization like that observed in a true attack.

The dry ice can produce such attacks in the whole body without the necessity of isolating the vertebral column. Thus in many experiments the cord was separated from the superior centers and the skin of the back elevated. The frog was placed on the edge of the table so that the posterior limbs remained free. The application of the dry ice on the back gave rise to violent attacks, with convulsions not only of the posterior, but of the anterior members and of the muscles of the body. The latent period is long. The reflexes did not return after the reheating, except in one case and then only in the anterior members.

When paraffin oil is employed as a bath, it is necessary to reduce the temperature extremely low in order to obtain an attack and that is probably due to the reduced calorific conductability of the oil. At  $6^{\circ}$  below no attack is produced. At  $18^{\circ}$  below the attack is feeble and entirely clonic.

In a bath of concentrated solution of NaCl, chilled below  $0^{\circ}$ , the attack is strong and presents a tonic phase.

4 *Means of inducing an attack at  $0^{\circ}$  or below  $0^{\circ}$*  It is possible to obtain an attack at  $0^{\circ}$ , or even below  $0^{\circ}$ , without exposing the cord by opening the vertebral canal. The first method consists of injecting a quarter of an hour before the preparation a certain dose of caffeine in every case insufficient for producing visible intoxication. Good results were obtained with one injection of 1 or 1.5 cc. of a solution of benzoate of caffeine in the lymphatic sac. The attack is produced up to  $3.5^{\circ}$ , showing fairly strong clonic and tonic contractions and being prolonged for 2 minutes. In one of these experiments it lasted 4 minutes. The latent period lengthened with the temperature of the bath: 45 seconds at  $0^{\circ}$  and 4 minutes at  $3^{\circ}$ .

The attack was also induced at  $0^{\circ}$  in North American frogs, but not in a steady and constant manner, if any other method was employed. The vertebral column containing the cord was first subjected to the action of an elevated temperature ( $28$  and  $36^{\circ}\text{C}$ ) for 2 to 5 minutes. Then the cord was plunged into a bath of Ringer solution at  $0^{\circ}$ . Under these conditions, a characteristic attack was observed in many cases, it was not strong, being confined in general to the clonic phase, with the latent period relatively short. In some cases, the attack itself was not produced, but the muscles showed clonic contractions strong enough to persist for 2 or 3 minutes. In a certain number of cases there was no reaction.

5 *Epileptiform attacks produced by chemical excitation of cord* The application of a crystal of sodium chloride on the medulla oblongata of the frog

periments sharp attacks occurred. The latent period varied from one to three minutes, the temperature of the cold bath being  $0^{\circ}$ – $0.5^{\circ}$ . This is a latent period much larger than that in the same conditions with *Leptodactylus ocellatus*.

The attack in *Rana pipiens* brought on by chilling after exposure of the cord, begins about one minute after the onset of chilling, by trembling fibrillations, which become stronger and stronger, there then follows a phase of disordered clonic contractions, and finally a tonic phase, the clonic contractions becoming additive from the contraction of the muscles. The attack is much more prolonged in the Brazilian frogs and in certain cases, endured for four minutes. It is almost always asymmetric, as is the case of attacks in European frogs, and contrariwise of those in *Leptodactylus ocellatus*. In one case at Yale the attack only commenced in the right leg when the convulsions disappeared in the left leg.

To sum up, the attacks were produced in North American frogs at  $0^{\circ}$ , or even a little below  $0^{\circ}$ , when the spinal column is opened and the cord is directly exposed to the action of cold.

3 *Attack produced below  $0^{\circ}\text{C}$*  In North American frogs attacks by quick cooling of the cord is always produced, if the temperature attained is sufficiently low and below the freezing point of water. The attack is not uniform and may show divergent characteristics depending on the method employed, which is probably due to differences in the rapidity of the chilling.

The application of ether, which is applied drop by drop on the dorsal surface of the exposed cord is often sufficient to produce an attack. Ethyl chloride is, however, less efficacious. In some cases the attack is almost exclusively clonic and lasts about two minutes. The tonic phase is absent or is feeble and reduced. After the attack, the reflexes return if the cord is again placed in a Ringer bath at room temperature. The application of dry ice on the closed spinal cord is marked by the production of extraordinarily intense attacks. The latent period is brief. Some seconds after the onset of chilling clonic contractions of muscles have already been initiated. The contractions increase and a period of tonic contractions follows, tetanic in nature and of great intensity. In general the attack is not symmetrical.

If, on the one hand, application of dry ice produces the phenomenon with exceptional intensity this method still is not entirely free of objection. The chilling is too extreme and cannot be applied with comfort. In consequence of this when the attack has ended, if one rewarms the cord by removing it to the circumambient temperature, the reflexes do not return; the centers have been functionally destroyed. On the other hand, the chilling may reach the nerves. In the Ringer bath at  $0^{\circ}$ , the nerves give no reaction, they are not excited by the cold. When ethyl chloride is employed, if a drop falls on the nerve, there are some quick muscular contractions and the cord loses its conductivity, but it is always possible to distinguish among the contractions those which are of central origin. With dry ice, chilling of the nerves across the other tissues may by itself give fairly strong contractions of the muscles.



elevated, as in those performed at Yale. The Ringer bath technique, at a temperature varying between 0.3 to 1° was employed. In one case, there was no attack properly speaking, but the clonic contractions were fairly strong and prolonged. In the other cases, clearcut attacks, although feeble, were produced. Naturally the application of ethyl chloride produced much stronger attacks. The attacks observed were similar to those which were found when the cord was subjected to temperatures approaching the upper limit. The temperature of 1 or 1.5° was then, in a similar manner, the actual upper limit of the attacks in *Rana catesbyana* in Cuba. If one compares these results obtained at Yale, where it was established that the attack was not produced in frogs, either at 0° or by application of ether or even by preliminary sensitization by caffeine, it may be concluded that there is a progressive modification, a slight adaptation of this sort of reactions of the central nervous system to conditions of climate. The fact that *Rana catesbyana* of Brazil did not have attacks at 0°, demonstrates that to induce this alteration needs several years.

### CONCLUSIONS

1. In North American frogs, *Rana pipiens*, an epileptiform attack by quick chilling of the isolated spinal cord was not produced at temperatures below 0°C, on the contrary they have been observed in South American frogs (*Leptodactylus ocellatus*).

2. However, the attack can be obtained at 0°C or a little below if the vertebral canal is opened and the cord directly exposed to the action of cold. In these conditions, the phenomenon, although frequent, was not constant.

3. The attack could be induced by cooling the cord below 0°C, by the application of ether, ethyl chloride, dry ice or by utilizing freezing baths of liquids with low freezing points.

4. The characteristics of the attack depended on the method employed. When the attack was complete, it was prolonged and presented clonic and tonic phases. The feeble attacks were exclusively clonic.

5. The preliminary injection of caffeine in the frog made attacks possible up to temperatures of 3.5°.

6. In many cases, the preliminary warming of the cord for several minutes at a temperature from 28 to 36° inclusive, made it possible to induce an attack at 0° or at least a little below 0°.

7. The direct application of a concentrated solution of sodium chloride on the spinal cord produced in the North American frogs a convulsive attack perfectly resembling that observed under the same conditions in the Brazilian frogs.

8. At 0° or below, *Rana catesbyana* in North America does not on chilling of the spinal cord have an attack.

9. This compared with the fact that frogs of the same species adapted to a warm climate (Cuba) for more than 20 years frequently with attacks at 1° below zero demonstrate that this convulsive reaction of the nervous sys-

produced a convulsive attack (9) Recently Ebbecke (10) also was able to induce convulsions by injecting into the cerebral hemispheres a concentrated solution of NaCl However, no one has been able to obtain convulsive reactions when the irritant substance acted directly on the cord (2, 9) Mous-satché and Vianna Dias (11) have shown that the direct application on the cord of a 20 per cent solution of NaCl provoked a strong convulsive attack in the Brazilian frog In the North American frogs examined at Yale concentrated solutions of NaCl put in contact with the cord have constantly given rise to an attack resembling perfectly those observed in Rio

6 *Experiments on Rana catesbyana* The specimens studied were of middle size, with weights varying from 300–500 g The muscles of the back were elevated in a manner to reduce as much as possible the surface to be chilled In no case did chilling at 0° induce an attack or any other reaction whatsoever In two cases, applications of ether were tried, but without effect With ethyl chloride a strong attack resulted, both clonic and tonic One of the frogs treated by caffeine, had no attack at 1°C, there were at most some trembling and clonic contractions The same frog, after a ten-minute exposure to cold had a slight reflex attack

#### DISCUSSION

Epileptiform attacks induced by rapid chilling of the cord appear in *Rana pipiens* in North America in a manner quite different from those observed in *Leptodactylus ocellatus* in South America They resemble rather the reactions observed earlier in European frogs Singly, the chemical excitations give reactions which are the same in *R. pipiens* and *Leptodactylus*, and which are different from those of European frogs

All these observations raise the question whether the proven differences are due to the fact that there are species differences, or to the action of climate and other conditions of life

The best means of studying this question is to acclimatize one species of frogs of one country to a different climate in another At Rio de Janeiro *Rana catesbyana* have been raised for several years being imported from North America In our laboratory it has been established that these frogs, born in Brazil, did not have attacks at 0° or below It was necessary to chill the cord below 0° by ethyl chloride, for example, in order to produce the attack Experiments were repeated every year to establish whether a modification of reactions could be found with the passing of time

On a recent visit to Cuba it was possible to do some experiments with specimens of *Rana catesbyana* From the information obtained, this species, also imported from North America, had been in Cuba for about 20 years, a much greater time than that which had elapsed since we had started to raise them in Brazil This made it possible to verify whether such a prolonged adaptation as this to a warm climate produced perceptible modification of the characteristics of the attack

In the experiments carried out at Havana, the muscles of the back were



tem changes slowly and progressively under the action of the surrounding temperature

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way, which is perhaps more important, is between the posterior hypothalamic region and the medial nucleus of the thalamus, through the periventricular system of fibers. It is known that lesions of the thalamus or the thalamo-cortical radiations also abolish the activity of the cortex (1, 2, 3, 9, 11, 7). We have also observed the disappearance of the spontaneous activity of the cerebral cortex by thalamic lesions or by section of the thalamo-cortical connections. Dempsey and Morison (6) have observed that even a small portion of cortex (1 cm sq), isolated by careful removal of all the cortex of the hemisphere and keeping its thalamic connections, can maintain its spontaneous bursts of activity. Besides, there is also evidence that stimulation of

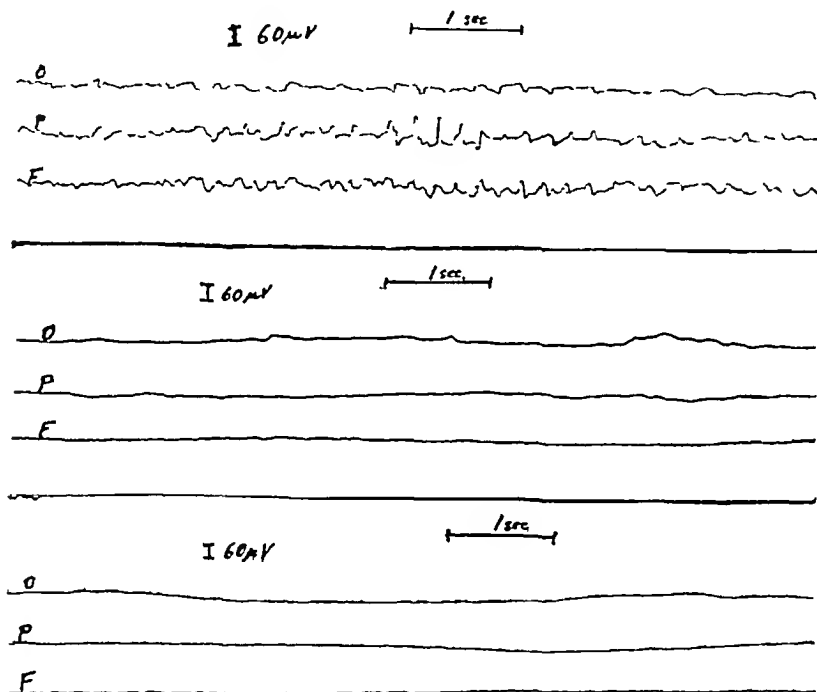


FIG 1 Spontaneous cortical activity before, immediately after and 50 min after lesion of the hypothalamus O occipital, P parietal and F frontal leads

the medial nucleus of the thalamus gives rise to widespread cortical responses similar to the bursts of activity of the spontaneous electrocorticogram (6, 7)

It can be assumed, therefore, that the hypothalamus may influence the cerebral cortex through its thalamic connections and perhaps by way of the medial thalamic region. The driving influence of the hypothalamus upon the cortex has been shown by experiments with hypothalamic stimulation (10). Cortical responses also occur after stimulation of the subthalamic regions (13).

# EFFECT OF HYPOTHALAMIC LESIONS ON ELECTRICAL ACTIVITY OF CEREBRAL CORTEX

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THERE HAS recently been growing evidence of the functional interrelation between the cerebral cortex and the lower subcortical levels. It seems that the cortical activity is sustained and influenced not only by the afferent impulses from the different sensory organs, but also by the impulses from lower subcortical levels (thalamus, diencephalon, etc.), that play a part in the maintenance and modification of the cortical activity. Exploration of the electrical activity of the nervous tissue has been used in the study of the functional interrelation between the cortex and the lower levels (6, 7, 9). We here report some experiments on the influence of hypothalamic lesions upon the electrical activity of the cerebral cortex.

## METHODS

The experiments were acute and carried out in 20 cats under Nembutal anaesthesia. The electrical activity was recorded from one hemisphere by silver wire electrodes inserted through small holes in the skull until in contact with the dura. The recording was bipolar with a distance of about 1 cm. between each pair of electrodes. Usually three pairs of such electrodes were used. The records were taken with a six-channel ink writing electroencephalograph (Adams and Bradley model). The hemisphere opposite to the one on which the records were taken was exposed by removal of the bone and through this side the different lesions were made. At the end of the experiment the brain was removed and kept in formalin for its macroscopical study.

## RESULTS

In the cat under Nembutal anaesthesia we have observed a spontaneous electrical activity made by waves with a frequency between 5 and 10 per sec. and a voltage of about 40 to 200  $\mu$ V. This spontaneous activity often appears in discharges of waves with a duration of several seconds (2 to 5) and repeats at intervals of 2 to 10 sec. Sometimes the spontaneous activity is more continuous. This type of spontaneous activity under barbituric anaesthesia conforms to the pattern found by different authors (2, 8, 14).

The lesion of the hypothalamic and basal portion of the brain completely abolishes the spontaneous activity of the cerebral cortex (Fig. 1 and 2), for the duration of the experiment (one or more hours). It is important to emphasize that this effect of hypothalamic lesions on the cortical activity takes place with the complete integrity of the thalamus and the rest of the brain\*.

The interrelation between the hypothalamus and the cerebral cortex seems to be through the thalamus. The anatomical evidence (5) shows two main pathways from the hypothalamus to the thalamus. One is from the mammillary bodies to the anterior nucleus of the thalamus. The other path-

\* This abolition of cortical activity does not seem to be secondary to changes in the circulation or blood supply of the cortex, because injection of stimulating drugs (strychnine, metrazol) brings back temporal and epileptiform discharges of the cortex.

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There is also clinical evidence to support abnormal slow waves in the cerebral cortex in cases of tumors involving the diencephalon and structures around the third ventricle (4, 15). It is difficult to find an explanation for these slow discharges and Adrian has suggested, in the case of Cairns, Oldfield, Pennybacker and Whitteridge (4), that the pressure on the thalamus by the tumor may have sent up abnormal volleys of impulses which might be responsible for the appearance of large waves on the cortex.

The lower levels below the hypothalamic and basal region of the brain do not seem to have such an intense and prominent influence on the cortical rhythms. The cortical activity persists, at least for some time, in the cat (2) and monkey (12), after completely sectioning the midbrain. In our experiments we have also confirmed the persistence of cortical rhythms, although diminished, after sections through the midbrain, even at the rostral level of the superior colliculi bodies.

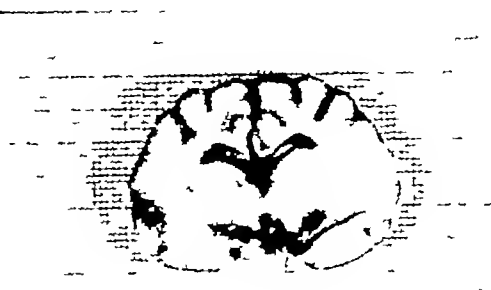


FIG 2 Section of the brain of the cat used in the experiment of Fig 1 showing the lesion of the hypothalamic and basal region of the brain

### SUMMARY

A lesion limited to the hypothalamus and basal region of the brain abolishes the spontaneous electrical activity of the cerebral cortex.

After lesions of the thalamus or its thalamo-cortical pathways, the cortical activity also disappears.

On the basis of different kinds of evidence it is suggested that the hypothalamus may influence cortical activity through its thalamic connections.

Section through the midbrain does not entirely abolish cortical activity.

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alter the frequency of discharge of the pacemaker neurons of the cardiac ganglion of *Limulus* to affect the over-all heart rate. The attempt is not made here to analyze the effect on the frequency of discharge of single cells within each heart beat.

#### MATERIALS AND METHODS

About three dozen adult specimens of *Limulus polyphemus*, mostly females, collected in Long Island Sound between March and June, have been studied in detail, by continuous electrical recording over periods of several hours. The specimens were kept in air at room temperatures for periods up to a week before use. The heart was exposed by removing the exoskeleton over it, carefully cutting the attachments of the pericardium. In most cases the organ was then removed from the body carefully and cleaned of pericardium and gonadal tissue. Lying on a horizontal glass plate, in air, at room temperature, the heart beat was remarkably steady in amplitude and frequency, for many hours. Usually no sea water, artificial physiologic solution or natural blood was used to bathe the heart. Little trouble was encountered from drying. It was preferred not to add complications of mechanical stimulation or change of chemical environment, and complete immersion was undesirable for electrical reasons. In some cases, however, it was necessary for special reasons to have a circumambient fluid or to wash the preparation. Both sea water and serum drained off after coagulation of the blood have been used. No differences between them have been noted.

The frequency and amplitude varied considerably between preparations. No correlation with condition of the animal, character of the dissection or other obvious factors was apparent. But both frequency and amplitude could be modified by conditions such as temperature, pH, state of distension, etc.

Certain experiments called for partial or complete removal of the ganglion. This was done by lifting the ganglion at one end and cutting beneath it with fine scissors carefully avoiding inclusion of muscle. If the dissection was carried far enough so that no pacemaking neurons were left attached to the myocardium (as tested by complete cessation of beat on cutting through the connection between ganglion and myocardium) not all of the heart but only the half or third of it nearest the undissected end continued to beat. If the separation was not carried so far the whole myocardium continued to beat but some portion of the ganglion capable of becoming a pacemaker was still attached to the muscle.

Polarization was accomplished by passing currents through the tissue by way of Ag-AgCl-sea water electrodes of convenient form. Leads came from the movable points of two parallel potentiometers across a series of dry cells. A galvanometer with a range switch permitting accurate readings of current flow from a few microamperes to milliamperes, was in series with one lead. A polarity reversing switch was included when desired. The current was introduced, increased or turned off by rotation of one of the stepless carbon potentiometers, although it was found that a sudden make or break was just as satisfactory and did not introduce special complications due to stimulation.

The electrical activity of the heart was recorded chiefly with moving coil, siphon pen oscillographs using Grass amplifiers (condenser-coupled) but many records were also made with the DC amplifier of Goodwin (12) and with moving coil, loop (Westinghouse) or cathode ray oscillographs. Three channels of recording were usually used simultaneously. Ag-AgCl-sea water electrodes in glass pipettes with cotton wicks were employed, several pairs usually being applied to the heart, each electrode mounted in an individual polystyrene holder.

Mechanograms were taken occasionally. This was done either by a thread which transmitted the transverse contraction of the heart to a hinged shutter intercepting a light beam falling on a photo-cell, or by inflating the heart with air and illuminating the photo-cell with a mirror mounted on a sensitive tambour in communication with the heart. Thus the mechanogram could be recorded by one of the amplifier-oscillograph channels on the same record as the electrical activity.

#### RESULTS

The character of the electrogram of the intact heart of *Limulus* is seen in Fig. 1 and 2. It is of complex form, most of the deflections are not dupli-

# ELECTRICAL POLARIZATION OF PACEMAKER NEURONS\*

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## INTRODUCTION

INDICATIONS are now numerous that the frequency of firing of the nerve cell depends on the DC electric field in which it lies ("electrotonic current," "somatic potential," "standing or steady state potential") Such DC fields are known to exist in nerve cell masses (19, 15) and evidence is accumulating that imposed constant currents will exert profound effects on the nerve cell. Direct evidence that imposed DC alters many properties of the nerve fiber has been obtained (14, 29, 13) Gerard (9) has reviewed the evidence that "cell discharges are related to cell potentials" (see also 14) A number of arguments have been brought forward to support the view that the cell body fires according to the over-all DC field resulting from the number, time relations, and spatial distribution over its soma of the axon action currents converging on it from many sources (10, 11)

A direct demonstration of a change in the frequency of firing of nerve cells by altering the intensity of an imposed DC field has been made by Barron and Matthews (5) in spinal motor neurons of the frog and cat The experiments of Auger and Fessard (3, 4), and Bradway and Moore (7) may at least include this effect Changes in the vertebrate electroencephalogram during and following the passage of direct current through the brain, have been reported (8, 19) Changes in frequency were not present or not prominent and in any case the nature of these waves and their relation to nerve cell firing is too little understood to allow interpretation of them in terms of individual nerve cell behavior or resulting axonal impulses

A definite effect on frequency of heart beat has been obtained in *Cladocera* (24), molluscs (2, 20, 22), and vertebrates (31, 23, 25, 30, 21, 16) But the effects are probably not exerted on nerve cells in any of these cases They differ in character from those here described since they are at least partially adapting

The ganglion of the neurogenic heart of *Limulus* seemed to offer an exceptionally favorable opportunity to test the question on nerve cells with an intrinsic automaticity The preparation is favorable for handling, dissecting and electrical recording The purpose of this paper is to answer the question whether an imposed constant current acting directly on the ganglion will

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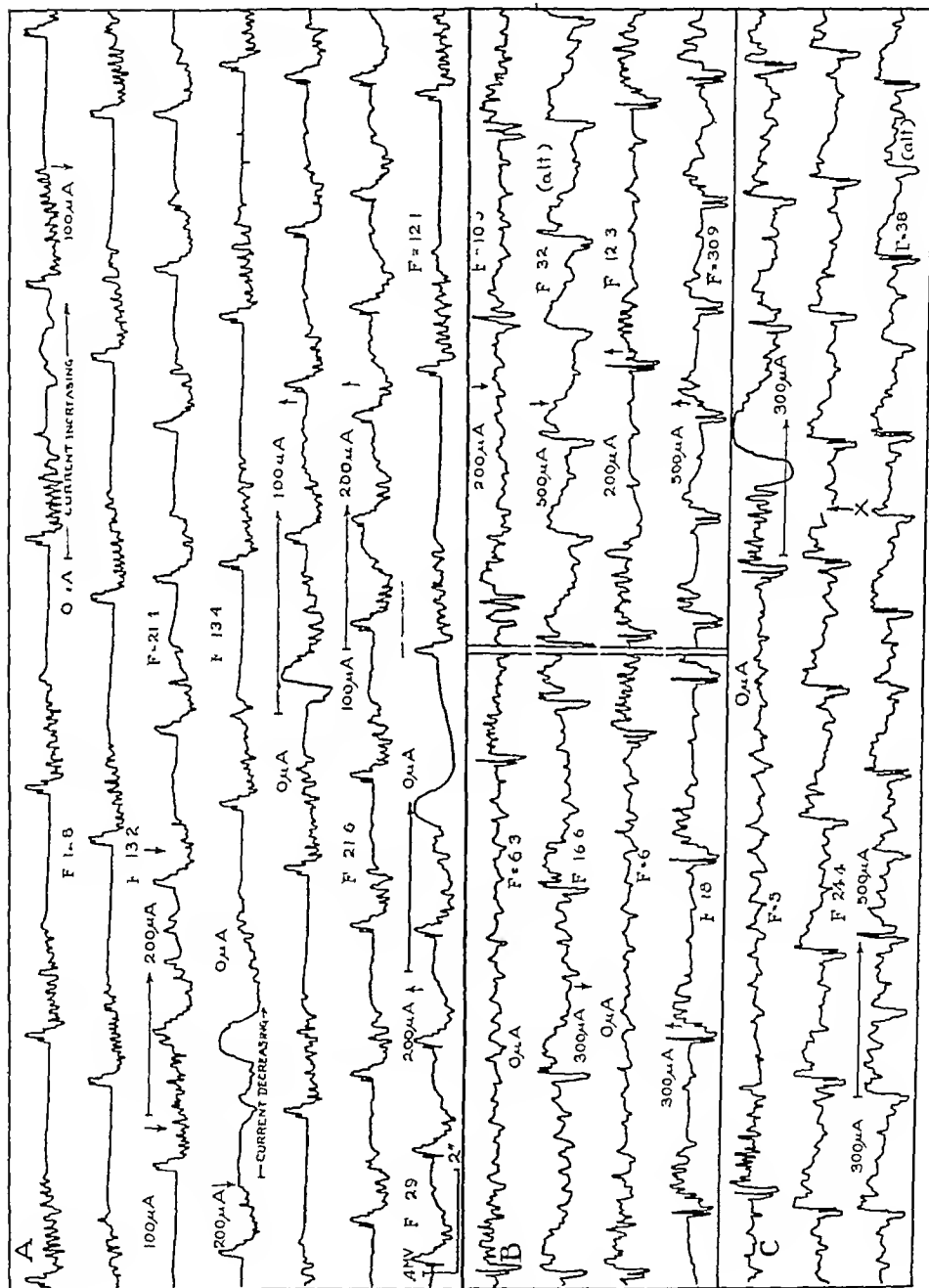


FIG 2 For legend see opposite page

cated exactly in successive beats, but the initial and largest waves are constant in form (over several hours, in a given preparation, from any one pair of electrodes) and the duration of the burst of electrical activity as well as the interval between beats is constant within rather narrow limits. The electrogram of the intact heart is probably to be considered as purely an electro-myogram. The electrogram of the ganglionic burst (Fig 1 and 5) displays

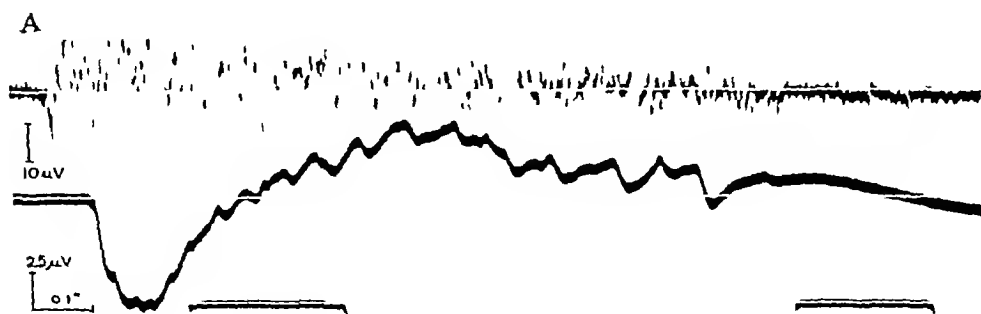


FIG 1 Electrograms simultaneously taken from (upper record) ganglion and (lower record) intact surface of the heart in a preparation similar to Fig 4. Grass amplifiers and Westinghouse loop oscillograph. Filters for upper record passing from slow (time constant  $\sim 0.3''$ ) to fast components. Filters for lower record passing from about 2 to 50 per sec. This upper limit is not responsible for the smooth line. Its character is not changed by switching to high pass filters. The fast activity of the ganglion does not show presumably because its amplitude is too small by reason of tissue shunting. Note the "DC" component in the upper record—a rapid rise and slow fall of the average potential. The slow component of the lower record has been attenuated to keep the excursions on scale.

If a mechanogram were shown its deflection would begin about 0.5 sec. after the initial deflection of the lower record and rise smoothly to fall after the electrogram has become quiet. A common observation, frequently more pronounced than in this record, is that the first indication of a heart beat is not a spike in the electroneurogram but a slow shift of the DC base line (cf. 6).

the same general constancies although the form and frequency spectrum of the component deflections are of an entirely different order (1, 17, 18, 26, 27, 28).

*Effects of polarization of intact heart.* In general, the result of passing small DC currents through the heart is an immediate, sustained, reversible change in frequency and character of the beat and the effect is graded with graded values of current passed.

The effect on frequency is the most obvious. Figure 2 shows a sample record and Fig 3, a plot of the heart rate against current passed for a typical experiment. It will be seen that the effect, in both directions of current passage, is an increase in frequency. This result is uniform throughout the experiments. No consistent and reproducible, indeed no considerable reduction in frequency, has been obtained with any placement of the electrodes. There may be slight but consistent differences between the effects of the

press, for the same heart may vary less than 1 per cent over many minutes or it may change as much as 10 per cent (rarely) in succeeding minutes. Ordinarily the number of beats in 20 sec have been counted and computed as beats per minute. The "resting" rate usually varies in the course of an experiment of several hours, including some relatively drastic polarization, by not more than 10–15 per cent. The frequency at a given current value is at least as constant as this and is ordinarily within the accuracy of setting the potentiometer.

Absolute values of current mean little because of the difficulty of defining the current density in the tissues. The points of greatest current density are probably the points of contact of the electrodes. The electrodes in these experiments were wicks whose diameter at the point of contact was two to three millimeters. Threshold currents were usually between 60 and  $150\mu\text{A}$ . A frequency increase somewhere near proportionality has in every case been realized between 150 and  $500\mu\text{A}$ . At some point between 500 and  $1500\mu\text{A}$  the increase is checked, the beat becomes small, irregular and difficult to count and may stop. But at any point in the experiment, even after apparent stoppage of the heart, interruption of the imposed current is followed immediately (*i.e.*, within the period of one beat) by resumption of the normal beat. There is often a slight deficit or excess over the initial "resting" rate and a gradual recovery over a period of minutes. (The lack of perfect reversibility in many preparations may be attributed in some cases to effects of the current—either fatigue or stimulation may occur, but in others it seems probable that a slow progressive change was taking place independent of the polarization. These effects were never great enough to overshadow those of imposed current and were often absent altogether.) With moderate currents the abrupt, maintained character of the effect and of its cessation is marked and the virtual absence of slow adaptation or stimulation after both "on" and "off" is characteristic.

Simultaneous with the change in heart rate during polarization is a change in the character of the beat. This may not be obvious in the mechanogram but is clearly shown in the electrogram of the intact heart. Moderate degrees of polarization result in slight or considerable changes in relative size of the initial and large waves of the electrocardiogram. There is usually also a reduced duration of electrical activity associated with each beat. Often a polarized heart will exhibit a wave form radically different from its "resting" wave form, none of the individual waves of the one form being recognizable in the other until the intermediate steps are studied. The wave form of the polarized heart may be quite different in the two opposite directions of current passage, though the frequency may be the same. With relatively high currents the electrocardiogram becomes smaller in amplitude, shorter in duration, greatly changed in form and, moreover, at a certain point, irregular. The irregularity may be only in form, so that successive beats are not alike, or in frequency, or both. Eventually as the current is increased, all these changes accumulate to render it impossible to count the

two directions of current passage in a given preparation and for a given pair of electrodes. But the character and direction of the differences were not consistent between preparations. Experiments reported in a later paragraph may provide an explanation of this inconstancy.

The heart in Fig. 3 was accelerated more than six-fold by moderate currents. This represents an increase almost exactly proportional to current for the "ascending" polarization, and more than proportional to current for the "descending" polarization. But the acceleration possible and the degree of proportionality vary among preparations, chiefly due to (i) the "resting" or zero-current frequency that happens to obtain in that specimen, (ii) the maximum frequency of which it is capable under the conditions of the experiment, (iii) any fatigue or gradual drop in "resting" frequency that is occurring, as well as (iv) its actual sensitivity to the imposed field. In these experiments the zero-current frequency has varied commonly between 5 and 18 beats per min., the maximum rate of beat of the intact (polarized) heart from 40 to 50, approximately. The constancy of the interval between beats was commonly better than 95 per cent. That of the number of beats per minute is harder to ex-

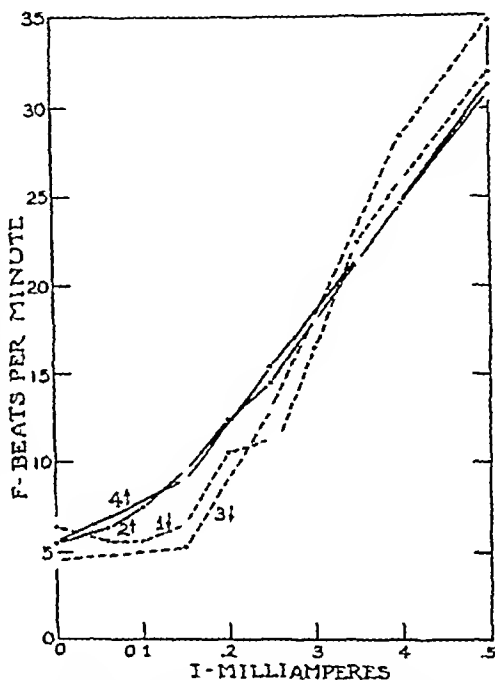


FIG. 3. Relation of heart rate to current imposed in a typical experiment. Intact excised heart polarized between fifth and seventh segments through cotton wicks resting on surface of heart. Points on curve obtained in succession from lower to higher currents and curves in the order numbered.

FIG. 2 (A) Electrogram of intact, excised heart under polarization, showing change in frequency of beat and in wave form of initial deflections. Note shorter duration of each beat as well as shorter interval between beats, during polarization. Currents turned on and off gradually (long arrows). Pickup from second segment of heart and sea water bath. Imposed current passed from fourth to sixth segment or vice versa, upward pointing arrows indicate "ascending" current (sixth segment anodal), downward pointing arrows "descending" current (sixth segment cathodal). Grass amplifier and ink writer. "F" = frequency of heart beat in cycles per minute calculated from measuring record.

(B) Another preparation: samples of a continuous record, similarly obtained. Note effect of polarization on wave form and different effect of the same current passed in opposite directions. Alternation of two wave forms at  $500\mu\text{A}$  "descending".

(C) Same heart as B. Polarization experiment some moments later showing abrupt character of change from one frequency and wave form to another. Continuous record about 20 sec. omitted at 'x'. Two alternating wave forms at  $500\mu\text{A}$ .

of the field, that the position of the second electrode would make little difference, providing it was situated where the high current density under it did not traverse a tissue of high sensitivity. That is "ascending," "descending," transverse or other axes between electrodes might be expected to have only slightly different effects. Further, it might be expected that much greater differences would be realized by changing the electrode over the sensitive tissue from an anode to a cathode, than by keeping it, for example, a cathode and moving the anode around it 360°.

To test these matters the heart was polarized with one electrode in a sea water pool broadly in contact with the heart, the other ("stigmatic")

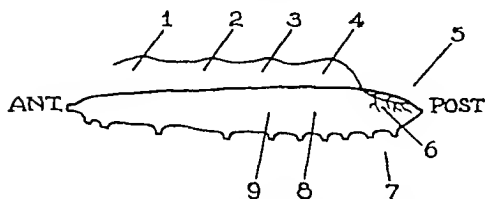


FIG 4 Diagram of the preparation with ganglion partially lifted off. The excised heart is carefully dissected to separate the ganglion, lifting it on wick electrodes, from either anterior or posterior end, leaving the other end attached to the heart. Ganglionic discharges continue to excite all of the myocardium reached by the nerves intact. With numerous electrodes in place (numbers correspond to text references), electrograms of the ganglion alone, the myocardium, the intact portion near one end and of regions within each of these may be recorded. Polarization may be imposed through any pair and the effects picked up at several points. The heart lies horizontally on a glass plate in a shallow pool of sea water or serum, not submerged. Electrodes 5 and 7 are in this fluid.

electrode touching the tissue. It became immediately apparent that a great difference existed between passing current from the stigmatic to the diffuse electrode and passing it in the opposite direction. The difference seemed to be one of effectiveness or threshold for the same modifications of heart activity. The cathode was the more effective stigmatic electrode. At a given moderate current level the stigmatic cathode commonly exerted eight to twenty times as great an effect in percentage increase in frequency as the stigmatic anode.

The lack of effect when polarizing through the lateral or anterior muscle or its high threshold relative to the region of the ganglion was clearly demonstrated. Slight differences were found between different

levels of the ganglion. It is probably this kind of difference which is shown by Fig 3—reversing the current is actually shifting the effective electrode (cathode) to a different part of the ganglion. If the direction of current passage is reversed not by reversing the polarity of fixed electrodes but by leaving the cathode in one place and moving the anode now anterior, now posterior, to it—or to the right or the left, close or far away, on the heart or in the sea water pool, no difference is found at moderate currents. The position of the anode is uncritical, that of the cathode is very critical as regards on or off the ganglion, and moderately critical as to the level of the ganglion.

*Polarization of ganglion and muscle separately.* A number of preparations of partial or complete separation of the ganglion from the myocardium were polarized in various ways. It was hoped to confirm the notion that the ganglion was the site of action of the imposed current and it was supposed that

beats, often paralyzing the heart. The mechanogram may be completely inactive and no contractions whatever visible upon close macroscopic examination of the heart while distinct activity in the electrocardiogram is still present. The heart is in diastole. At still higher currents when the electrocardiogram has ceased activity, its DC level is that of rest, not a smoothed out tetanic series of spikes. With somewhat lower currents while the mechanogram still follows the electrocardiogram it may lose amplitude before the electrical record, the contractions may become local, various parts of the heart not being synchronized, the mechanogram often skips beats and has been recorded for a long period responding to every third, fourth or fifth beat of the electrocardiogram. (It should be recalled that the electrocardiogram under discussion throughout this section is supposedly an electromyogram.) Commonly, at a certain high or even moderate current value, the succession of heart beats suddenly exhibits an alternation between two regularly recurring consistent wave forms. One of these is usually recognizable as the previous sole wave form, the other being entirely new. Often the frequency curve shows no break at this point but continues to rise steadily (see Fig. 2). Every stage in this series is immediately reversible, the original wave form being perfectly restored on cessation of polarization, even though many minutes have elapsed since that form was last exhibited. Moderate degrees of polarization can be maintained for long periods (30 min.) with perfect reversibility of wave form and only slight loss or gain in frequency.

All the effects just described were obtained by polarizing longitudinally from one electrode on the mid-dorsum of the heart to another more anteriorly or posteriorly. The attempt was made to discover the influence of the location of the electrodes. It was found that moving either or both electrodes forward or back along the mid-dorsal line had only a slight effect on threshold, within the segments of the heart occupied by the ganglion. Moving both electrodes away from the ganglion, however, for example one into the first segment, the other far laterally in any segment, resulted in a large change in threshold, raising it so that even currents of 500–1000  $\mu$ A only slightly or not at all increased the heart rate. The same was true if both electrodes were dipped into sea water pools in contact with the heart, restricted such that the current had to pass through the heart, whether the electrodes were on opposite sides of the heart, right and left, or anterior and posterior to its ends or in any other position. The suggestion arose therefore that the effect of imposed currents as applied earlier was a local one dependent on the points on the heart touched by the electrodes, these points having the highest current density, and various regions of the heart being differentially effected by the same current density. If this is correct, several interesting implications would seem to follow. Since the heart tissue and the film of salt water on its surface are good conductors, the current density will be highest at the point of contact of the electrode and will rapidly fall off with distance therefrom, the current path widening in a cone-like fashion. This might mean that one has little control over the orientation of the effective portion



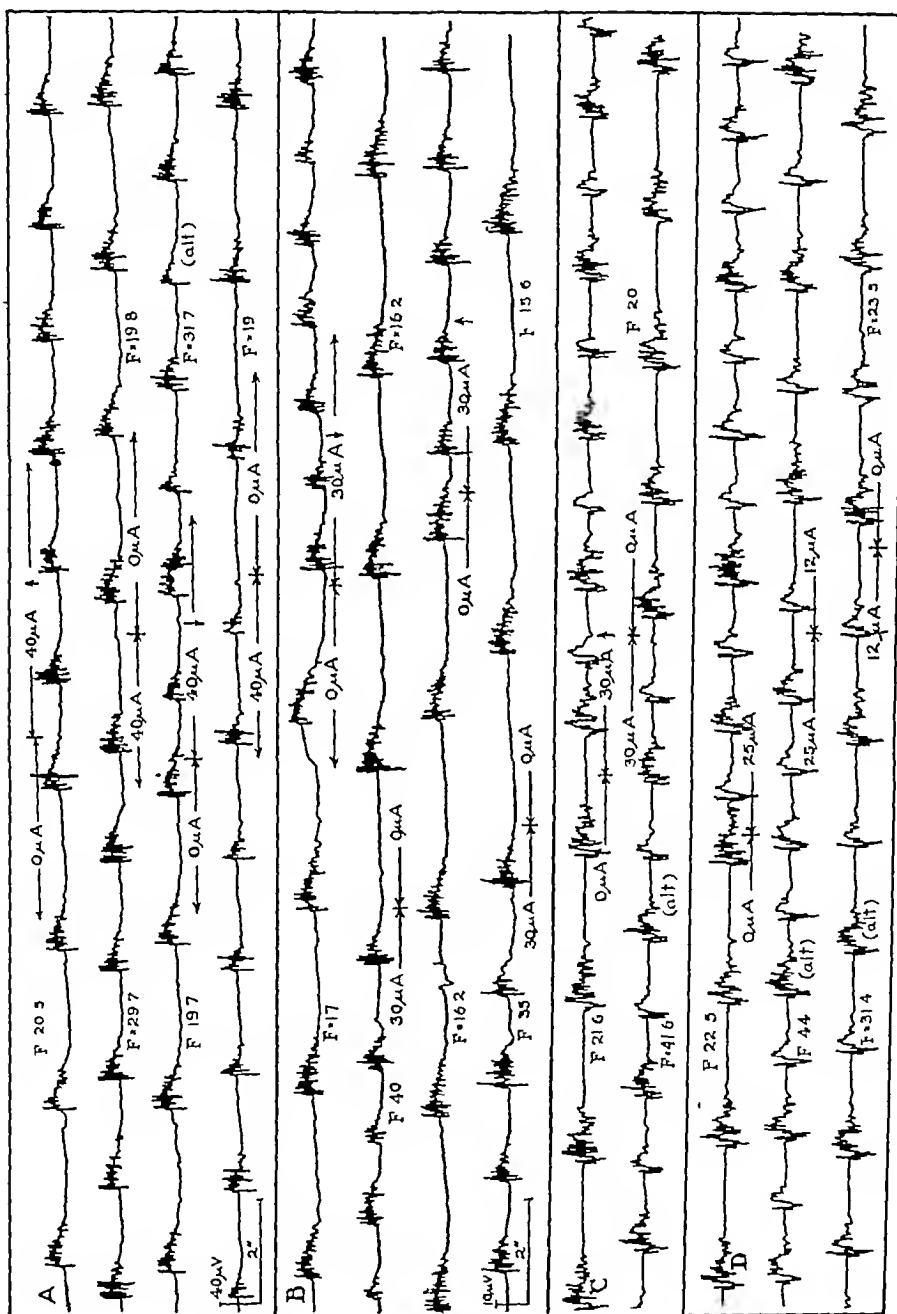


FIG. 5 For legend see opposite page

the dimensions of the ganglion—about those of the wick electrodes—would, by confining the current path, provide a more nearly constant current density through some length of the ganglion and perhaps reveal differences between “ascending” and “descending” currents distinct from the effect of the position of cathode or anode

The first question was easily answered when polarization of the ganglion-free or near ganglion-free muscle produced no effect on heart rate or wave form up to currents of several milliamperes, whereas  $20\mu\text{A}$  through the ganglion along, or only a small portion of it, sufficed to speed up the beat of that same muscle by fifty per cent or more (A diagram of the preparation discussed in this section is given in Fig. 4) Cutting across the last nervous connection between ganglion and myocardium, but leaving the cut nerve in contact with the muscle—thus interrupting physiological but not electrical connection—obliterated the effects on the muscle. But leading off from the ganglion, its discharges could be recorded and the polarization effect seen to be still present in them

The problem of polarity was more difficult. Under the conditions of the experiments it was possible to show that neither cathode nor anode was consistently more effective and that direction of current flow did not have a consistent effect. Polarization was effected through various combinations of the electrode positions shown in Fig. 4 and the electrogram of the ganglion or/and muscle led off from other pairs not lying in the current path. Threshold currents were between 5 and  $12\mu\text{A}$ , marked acceleration occurring at  $20\mu\text{A}$  and many preparations exhibiting the signs of high current described above, at 30 or  $40\mu\text{A}$ . All the phenomena described for the electrogram of the intact heart were demonstrable in the high frequency ganglionic bursts as well. Consistency of major features of wave form, alternation of two definite wave forms at high currents, shorter bursts at higher frequencies, and the abrupt, reversible character of the effect are some of these (Fig. 5)

When the polarizing electrodes were any two of electrodes 1–4 (Fig. 4), thus both on the ganglion, there was, in some cases, a difference in the frequency at a given current level when the polarity was reversed. This, of course, may be due to the different position of a more effective electrode as in the intact heart or to the direction of current flow through some critical region of the ganglion. But in most cases there was no appreciable difference. When one electrode was in the circumambient sea water (5, Fig. 4) and the other was on the ganglion (1, 2, 3 or 4, Fig. 4) the small currents necessary to effect the lifted portion of the ganglion were far below the threshold for any ganglionic tissue still on the heart, or for the myocardium. The results of polarizing in this way were distributed among all the possible results. In some cases there was a greater effect when the electrode on the ganglion was the anode, in some cases when it was the cathode, in the rest there was no significant difference in effect when the current was reversed. As with the intact heart, such differences as were found were in threshold and degree of acceleration, a reversal of effect, i.e., deceleration, was never encountered

pacemakers exist in different parts of the ganglion, presumably with different anatomical orientations, it is quite conceivable that any decelerating effect of a given DC field would be exerted only on certain cells and would be masked by the accelerating effect of the same field on other cells, which would then take over the pacemaking function. Thus a new pattern of cells would initiate the heart beat and this may account for the difference in wave form of the electrocardiogram during polarization and for the difference in wave form at the same frequency with opposite current polarities.

### SUMMARY

1 The isolated heart and cardiac ganglion of *Limulus* has been used as a test of the hypothesis that the frequency of firing of nerve cell bodies depends upon the DC electrical field in which they lie.

2 It is shown that a direct current passed through the intact heart results in an abrupt, sustained, reversible increase in frequency of heart beat and in changes in wave form of the electrogram.

3 The effect apparently is exerted locally, at the point of contact of the electrodes and the tissue, where the current density is highest. It is usually confined to the cathode in preparations of the intact heart. The anode and the gross orientation of the field appear to be uncritical. In preparations of the isolated or partially isolated ganglion the cathode was not consistently more effective.

4 The effect has been obtained only in the direction of an increase in the normal frequency. A possible explanation of this is offered.

5 It is concluded from the abrupt non-adapting, non-accumulating and promptly reversible character of the effect, that it is a direct effect of the DC field.

6 The site of action is apparently the ganglion, more specifically the region of the pace-making cell bodies.

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## DISCUSSION

It seems probable from the evidence presented that the effect of an imposed direct current is a direct result of the electrical field itself. The abrupt character of the change in heart activity at "on" and "off" as well as the steady (non-adapting and non-accumulating), character of the effect during current passage argue against an indirect result of such factors as temperature rise, or products of electrolysis. Whatever the mechanism, it is one which comes to equilibrium within a fraction of a second. Furthermore it may be characterized as increasing up to a certain point directly with the current, apparently a function of current density, *i.e.*, field intensity. It can act locally, altering the activity of the whole heart by acting on a few cells. And it apparently does not alter the intrinsic electrical field or any other property that cannot return to its original state in a fraction of a second.

The site of action seems to be the ganglion and apparently may be some critical point in the ganglion. Any possible changes in peripheral nerve or muscle do not seem to enter into the effect as measured in these experiments.

There remain the questions why no consistent relation to gross anatomical orientation is evident and why the effect is obtainable in only one direction, *i.e.*, increased frequency. The evidence from the present experiments does not afford an answer but it may be that there are consistent relations with the microscopic anatomy of the ganglion. The histology is insufficiently known but it seems more than probable that the nerve cells are oriented in all directions. If this is so and the actual effect of the DC field is determined by its orientation in relation to the polarity of individual cells then one might expect no consistent relation to gross anatomy. Moreover, since it is evident that the pacemaker of the entire heart may be quite localized—perhaps one cell—and that it may shift, that is, many potential

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FIG 5 Electrograms of the ganglion of a preparation similar to Fig. 4. Grass amplifier and ink writer. The fast components of each burst of activity are not recorded faithfully by the ink writer (see Fig. 1) but the time relations and slow components are, down to about 2 per sec. Electrode numbers correspond to those of Fig. 4.

(A) Polarizing between electrodes 3 and 4, pickup 1 to 2. Current made and broken abruptly. First two lines consecutive, 80 sec. omitted between lines 2 and 3, 70 sec. between lines 3 and 4. Note abrupt maintained change in frequency and duration of bursts at make and break of currents.

(B) Same preparation, polarizing between electrodes 4 and 5 so that a short segment of ganglion becomes alternately anodal and cathodal with respect to a diffuse electrode, as current is reversed. First two lines consecutive, last two lines consecutive, 2', 40" omitted between lines 2 and 3.

(C) Same preparation, polarizing between electrodes 1 and 2, pickup 4 to 5, showing alternation of two types of burst. Two lines consecutive. Calibration as in B.

(D) Same as C. Three lines consecutive. Alternation at 25  $\mu$ A appears to double the frequency simply by adding a new (simple, shorter) burst midway between existing ones, but at 12  $\mu$ A, overall frequency still higher than normal, the bursts are still evenly spaced, thus the 'original' or any one, wave form is actually slower than normal. The frequency determining mechanisms must control the overall frequency rather than simply adding extra beats or starting a new independent pacemaker.



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substituted by a fine thread of cotton soaked in saline, it was clearly a potential originating at the electrode-drug liquid junction. Such potentials and also the electrode polarization could be minimized by the use of silver-silver chloride electrodes making contact over about 1 mm fibre length by a fine thread of cotton wool soaked in Ringer (27). Sometimes the greater part of the applied drug soaked into the cotton wool wick and a variable dilution came into contact with the muscle fibre. Usually, however, the drop could be applied and kept under the hook while the recording was done. Application of such a drop effectively broadens the contact area of the recording electrode to about 2 mm. Thus the contact is not very well defined and it is not possible to restrict recording accurately to such a very small area as the endplate. Estimation of the actual size of potentials at such small regions can be made by comparison with the sharply localized interface leading (25, 26).

Photographic records were taken on a slow falling-plate camera or by using a thyatron time base.

(ii) When long lasting potential changes after drug application were measured, the following method was employed in isolated nerve-muscle fibres and in the whole sartorius. The test solution was put into a small glass dish, which in its turn was placed in a bath of paraffin oil. The muscle was held by two forceps in a vertical position, while still in paraffin, and was lowered into the dish underneath containing the test solution. Thus the muscle could be immersed to a required degree, while one electrode was kept on the muscle in paraffin, the other being in the drug-saline solution. This latter electrode would record changes caused by the drug action at the saline-paraffin interface. The potential which developed was recorded by non-polarizable silver-silver chloride electrodes (as above) and could be balanced by addition of a constant potential to the recording circuit. When in the balanced condition a shortcircuiting key was placed across the input and then opened, no deflection resulted. A constant potential input of 50 mV showed no appreciable alteration during 30 to 40 min continuous application into the recording circuit. Hence, electrode polarization would not seriously distort the potential readings.

The above method was also suitable for the investigation of potentials along the normal fibre, especially around the nerve entry. But usually the whole nerve-muscle fibre was lifted into paraffin while one electrode remained at one end of the muscle fibre and the other was moved to different positions. In the early experiments potential changes were found, but these were due to alterations in grid current in the input stage which varied inversely with the preparation resistance, as the interelectrode distance was altered. This was eliminated by the use of a preamplifier of high input impedance. For the denervation experiments the sciatic was cut about 1 cm above the thigh and the frogs were kept from 2 to 11 weeks at room temperature (16–20°C) and were fed twice a week by insertion of meat, usually liver, into their mouths. Successful dissection of single muscle fibres was more difficult as injury due to manipulation seemed to occur more frequently and fibres did not recover as readily after treatment with the various drugs.

In the single fibre preparations from denervated muscle the location of the endplates could not be discerned under the microscope. In the normal muscle, however, the endplate free regions can be established with reasonable certainty during the dissection. Usually all the fine nerve branches are well visible under suitable illumination and generally they innervate bundles of muscle fibres over a small circumscribed area (cf multiple innervation in isolated muscle fibre preparations 27).

Altogether 65 preparations were successfully experimented on during the present investigation, 23 were dissected nerve muscle fibre preparations. Sometimes the latter were used on 2 successive days and were kept over night in Ringer at a temperature of 10–15°C. Acetylcholine B D H was used in all experiments. The different KCl concentrations were obtained by diluting in Ringer KCl solution isosmotic with it. One per cent pure nicotine and one per cent caffeine stock solutions were employed.

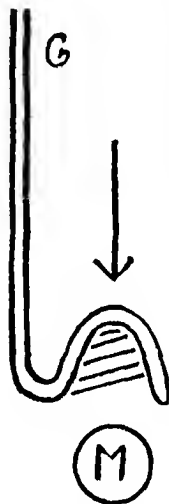


FIG 1 Diagram illustrating local application of drugs to an isolated muscle fibre M (transverse section). G, glass rod terminating in a fine hook. Shaded area shows droplet of the drug which is brought into contact with the muscle fibre by lowering the glass rod (arrow).

# SPECIFIC EXCITABILITY OF THE ENDPLATE REGION IN NORMAL AND DENERVATED MUSCLE

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## INTRODUCTION

SPECIFIC properties of the endplate region have been described by Langley (28) who showed that small concentrations of nicotine excited muscles around the nerve entry, while much higher concentrations were required for a similar effect on other regions. The "receptive substance" was regarded as possessing different properties from nerve and muscle. Lucas (30) found a distinct electric excitability of the neural region in the frog's sartorius—the  $\beta$  excitability—but this could not be shown by other investigators (33).

In a series of experiments in normal and chronically denervated muscle, drugs were applied to the 'nervous' and nerve-free regions of the whole sartorius and to the endplate and other parts of single nerve-muscle fibre preparations (25), while the potential changes at the place of application were recorded. It seemed of special interest to investigate the effects of acetylcholine and potassium besides other substances as nicotine and caffeine.

Some authors (11, 12) found longitudinal potential gradients in resting muscle fibres and also potentials localized at the end-plate region. Special properties of the endplate itself could be found in the present investigation but with the technique employed, no appreciable longitudinal potential gradients were detected in uninjured resting muscle fibres, either localized around the endplate regions or on other parts of the muscle.

## METHOD

Frog's sartorius or isolated nerve-muscle fibre preparations from the M adductor longus of the Australian *Hyla aurea* were used. The potential changes were recorded with a resistance-capacity coupled amplifier, the response usually falling to one half in about 2.0 sec, occasionally 5.0 sec, when large coupling condensers were used. Although the potential changes after local application of drugs (Section II A, 1) lasted for several seconds, only those taking place during 0.2–0.5 sec. after application are discussed here.

*Technique of drug application.* (1) The single nerve-muscle fibre preparation was lifted into paraffin while two electrodes made contact with it. One electrode was usually on the end of the muscle fibre while the other could be moved to different positions with the help of a micro adjustment. By means of a small glass hook containing less than  $0.3 \mu$  l. of solution drugs were applied under direct microscopic observation (Fig. 1). When the recording was done from the region of application, the drugs were put on the tip of one recording electrode in contact with the muscle. The small drop could be held there in position by the glass hook. When platinum electrodes were used, the application of ACh or KCl produced an initial positive potential. Since this also occurred when the muscle fibre was



well with Buchthal and Lindhard's (13) findings on lizard's motor endplates. Other drugs, as nicotine, KCl and with some exceptions caffeine, which sometimes failed to set up impulses, showed the same selective excitatory action at the endplate region. Figure 2Aa illustrates a train of impulses set up after application of  $10^{-6}$  ACh to a motor endplate of an isolated single muscle fibre. An initial negativity of the endplate is observed to lead up to the origin of the first spike, and each successive spike seems to be initiated again by a similar potential which is built up again after the refractory

period of the preceding impulse. If the same or even a much higher concentration of ACh is applied to a spot a few mm from the endplate, practically no potential is recorded from the region of application.

A critical depolarization of the muscle membrane by the endplate potential (e.p.p.) (18, 25) or by an applied constant pulse (27) is known to set up muscle impulses. It is safe to assume that a similarly critical depolarization takes place here after application of ACh or the other drugs (see discussion). This view is further supported by the effect of subthreshold concentrations as seen in Fig. 2Ab. There a slow negative potential only is set up. With increase of concentrations larger potentials are set up until at a critical value a spike arises at the height of the potential. The depolarizing action of the drugs does not

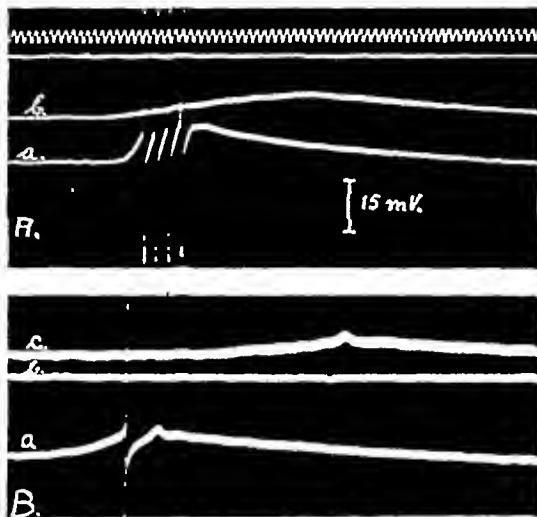


FIG. 2 Local application of acetylcholine to the endplate region of a single nerve-muscle fibre preparation. Recording is done from the point of application. A, a,  $10^{-6}$  ACh sets up four propagating muscle impulses; b, a weaker concentration of ACh produces a local negative potential only; B, c,  $10^{-6}$  ACh sets up a local response at the height of depolarization, a, after a stronger concentration of ACh a spike is initiated and following it is a similar local response as above; b, saline application. Time scale 1 div = 10 msec (see text).

cease after the impulses have been set up, but decays slowly over many seconds as can be shown by recording with an amplifier of a long time constant. This is presumably due to the slow dissipation of the drug action.

With concentrations near threshold frequently small monophasic potentials (abortive impulses) have been recorded at the endplate region (Fig. 2Bc). A slightly higher concentration sets up a propagated impulse and following it is a small potential similar to that seen above (Fig. 2Ba). Such local responses can be set up in isolated normal muscle fibres by electric

## RESULTS

## I MEASUREMENTS OF POTENTIAL DIFFERENCES ALONG MUSCLE FIBRES

In 10 single nerve-muscle fibre preparations careful measurements of potential differences were made between various points on the surface of uninjured muscle fibres. The innervation of the muscle fibre was preserved and the endplate was clearly seen. The location of other endings on the same muscle fibre could be established in most preparations (cf. above). The condition of the preparation was checked frequently by recording at different positions the muscle action potentials set up by nerve stimulation. The action potential size varied in different preparations and was generally between 50–100 mV, depending on the shunting effect of tissue rests along the fibre. At the end of the experiments the muscle was usually injured by crushing or by KCl application and 'resting potentials' were recorded. These were smaller than the spikes in single fibre preparations. In all preparations random potential differences of 1–2 mV were observed between different parts on the uninjured muscle fibre. In 3 experiments potential differences as high as 4–5 mV were recorded but in each case these potentials were not related to the known endplate. All these preparations might have had slight injuries, but they continued to contract normally for 1–2 hours after the potential measurements were taken.

In 5 experiments the measurements were repeated about 12 hours after the muscle fibres had been 'killed', in these the protoplasm was 'broken up' and, as expected, no potentials at all could be detected.

In frog's sartorius the endplates are usually located in foci several millimeters from the nerve entry (24). There, potentials around the endplate region—if present—should be recorded. However, in 20 preparations in which subsequently potentials due to drug application (Section II) were measured, no potential differences around the endplates were found under resting physiological conditions. Thus large potential differences along the surface of muscle fibres especially between the endplate and the surrounding muscle as found by Buchthal and Lindhard and also others (cf. monograph 1939) could not be detected in frog's muscle (but see discussion).

## II A NORMAL MUSCLE

(1) *Local application of drugs and recording from nerve muscle fibre preparations.* When ACh is applied to the endplates directly (13) or injected into the circulation (9, 5), it has a powerful stimulating action on muscle fibres. Application to the surface of the sartorius sets up a train of impulses, the threshold concentration being at least 10 times higher at the pelvic end than elsewhere along the muscle (19). The stimulating action of ACh at the endplates was readily confirmed in the isolated nerve muscle fibre preparations, but no impulses at all could be set up at other regions. Even 1000 times the threshold concentration at the endplate failed to produce more than a small local contracture at an endplate-free region. This agrees

tions the size of the spikes, set up by nerve stimulation, was frequently recorded. When the whole muscle fibre was immersed into 0.22 per cent KCl nerve stimulation became ineffective after 1 or 2 min. After lifting part of the preparation, comprising the nerve-muscle junction, into normal saline, the gradual recovery could be observed under the microscope. Nerve stimulation is followed first by a local contraction around the junctional region, then the contraction may gradually spread along the rest of the fibre which is not immersed, while the part in KCl does not contract. This shows that the process of neuromuscular transmission recovers before the muscle is able to conduct impulses. It also suggests that abolition of indirect excitability after KCl application was primarily due to the effects of potassium on the muscle fibre itself and not on the process of neuromuscular transmission.

A dose of curarine which blocked neuromuscular transmission diminished the sensitivity of the endplate at least 10 to 100 times to ACh, nicotine and caffeine. Sometimes no impulses at all could be initiated and doses such as  $10^{-3}$  ACh set up only local contractures when applied to the endplate region. Usually the sensitivity could be restored after soaking the preparation in normal saline. The threshold concentration of KCl was frequently increased by curarine but to a much smaller degree than with the other drugs.

Two sartorius preparations were isolated together with the ventral roots. Stimulation of the ventral roots resulted in full contraction of the muscle and a normal action potential could be recorded from it. During application to the endplates of ACh, KCl and nicotine in concentrations well above threshold for setting up repetitive responses, no potentials could be recorded from the ventral roots. It can be concluded therefore, that the motor nerve endings of frog's muscle are not excited by these drugs. In cats, however, nerve discharges following injection of ACh were found by Masland and Wigton (31).

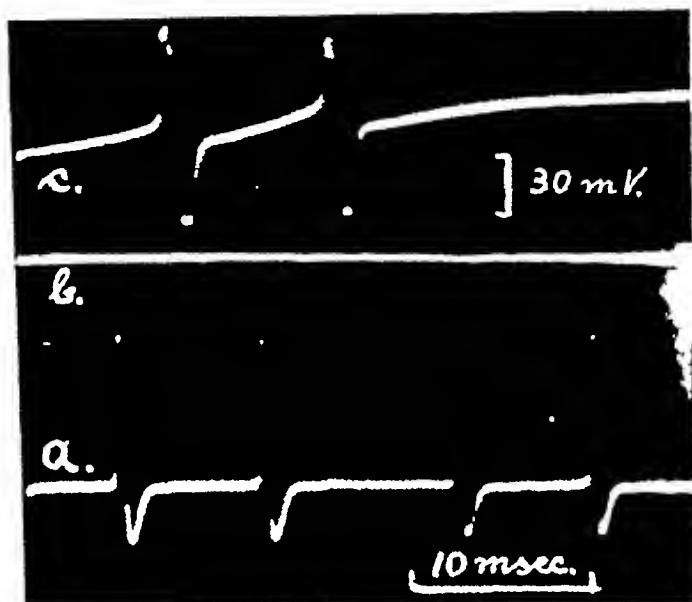
(2) *Change of "resting potential" during drug application.* It seemed of interest to record changes caused by long lasting application of drugs to different parts of the whole sartorius or to single muscle fibres. The preparation, set up vertically, was lowered to a required degree into the test solution. All the potential changes thus measured are caused by the depolarizing action of the drugs on the muscle membrane and are relative to the lead on the 'normal' muscle part in paraffin (cf. Method). At different intervals after immersing part of the muscle into the test solution changes in potential are measured.

No appreciable potential, (less than 1 mV) appeared when the endplate-free part of a nerve muscle fibre preparation was lowered into a threshold concentration of ACh for 10–15 min. (Threshold concentration of a drug was the concentration which set up impulses at the endplate when applied locally by a fine glass-hook.) However, when the endplate reached the test solution, a train of impulses was set up and a potential of several millivolts lasting for at least 10 min. could be recorded at this spot. When a muscle

stimulation or in relatively refractory muscle by a nerve impulse (26, 27) Immediately after a spike (Fig 2A) the potential returns to the original base line only and the negativity is then built up again This seems to be analogous to the 'collapse' of the endplate potential, or of an applied catelectrotonic potential, during the muscle spike and to its rebuilding after the refractoriness has passed off (18, 27)

When a train of impulses is set up at the endplate as in Fig 2A, 3c, and

FIG 3 Potentials recorded from nerve-muscle fibre preparation c, acetylcholine applied to endplate while the recording is done from point of application (as fig 1, but faster sweep) a, impulses initiated at endplate are recorded 5-6 mm away from it Note the absence of a negative potential preceding or following the spikes b, base line



the recording is done a few mm away from the point of application, no negative potential precedes or follows the spikes (Fig 3a), only 'simple' propagating muscle impulses are recorded and they are identical whether set up by nerve stimulation, electrical stimulation or drug application

Threshold concentrations varied considerably with all the drugs, probably depending on the condition of the individual fibre and on the accuracy of application to the endplate ACh and nicotine thresholds were at about  $10^{-6}$  while the caffeine threshold was mostly 10 times lower 0.22-0.45 per cent KCl diluted in Ringer from an isotonic stock solution gave usually threshold excitation At times no propagated impulses could be set up by local caffeine application, and with high concentrations ( $10^{-3}$ ) only contractions were "washed" by applying drops of Ringer which could later be removed by a small pipette But even so, after several applications of the drugs, frequently no further impulses could be set up After lowering the preparations into saline the fibres usually recovered in a few minutes Between applica-

test solution and the developing potential was measured again. Eserine had little or no effect on the nicotine and caffeine potentials.

In view of the selective excitatory action of KCl on the endplate, a difference in its depolarizing effect on the nervous part or pelvic part of the sartorius might be suspected. Two methods were employed in this investigation. (a) The muscles were immersed into different concentrations (0.22, 0.45 and 0.89 per cent) of KCl, the pelvic end first and then the nervous part while the development of the depolarization potential was plotted. Thus Fig. 4B shows the potential developed at the pelvic end (full circles) and at the "nervous" part (crosses) after immersion into 0.22 per cent of KCl. The differences do not seem significant. (b) The pelvic 2-3 mm. and 5-6 mm. of the tibial end were lowered simultaneously into two separate small dishes containing the same concentration of KCl, into these dipped the two recording electrodes. The two small dishes were in a big dish containing paraffin and were bridged by the middle of the sartorius, held up in the paraffin by two glass-hooks. Potential differences of a few millivolts were sometimes found over the first 10 min. but they usually disappeared after 20 min. Curarine did not alter the potentials in any way comparable with its striking effect on the other drugs.

#### B. CHRONICALLY DENERVATED MUSCLE

After about two weeks the sensitivity of denervated muscle is considerably increased not only to ACh (6, 10), but also to nicotine and caffeine application. The KCl sensitivity, however, is not appreciably increased if compared with that of the not denervated leg of the frog. After denervation from 7 weeks upwards concentrations of the drugs as low as  $10^{-10}$  or  $10^{-11}$  set up impulses at the endplate regions and even a slight mechanical stimulus can initiate bursts of impulses in such a denervated sartorius. The muscle is practically on the verge of the spontaneous activity which was recently observed by Reid (32).

The isolated muscle fibre preparation gave a good opportunity for testing the sensitivity of the endplate-free regions in denervated muscle. No endplates can be observed in such preparations and therefore the whole length of the muscle fibre had to be tested. With some parts of the fibre the threshold concentration to set up impulses was 100 to 1000 times higher than with other parts. It seems safe to assume that these less excitable regions of muscle fibres are endplate-free regions. This is strongly supported by the invariably lower excitability at the pelvic end of the denervated sartorius. For example, in some instances the ACh sensitivity was increased on the 'nervous' part of the muscle to  $10^{-11}$  as compared with  $10^{-8}$  on the normal side. With careful application to the pelvic 1-2 mm. of the muscle in some of these experiments the threshold concentration was  $10^{-7}$  or  $10^{-8}$ . Differences in sensitivity even to mechanical stimulation with a small glass rod or platinum loop were quite marked between the pelvic end and the rest of the sartorius especially after denervation of more than 7-8 weeks.

fibre was lowered into 0.22 or 0.45 per cent KCl solution an immediate potential change was caused and became constant in 2-3 min. This occurred wherever KCl surrounded the fibre. No difference between endplate and endplate free parts was noticed. Whenever this potential exceeded a few millivolts, the propagating spike was diminished or abolished altogether (cf similar findings in squid giant axons where true resting potentials across the membrane could be measured, [16])

Also in the whole sartorius preparation the depolarizing action of ACh, nicotine, and usually caffeine could be well demonstrated. When the pelvic 2-3 mm of the muscle was lowered into the test solution of just threshold concentration, no potential developed there, and no contraction at all was noticed. With further immersion of the muscle, however, fibrillation was set up and persisted for several seconds. At the same time a potential developed, reaching about 8-10 mV in 15 min (cf Fig 4a). Sometimes this potential was very small (1-2 mV) and it always varied at different

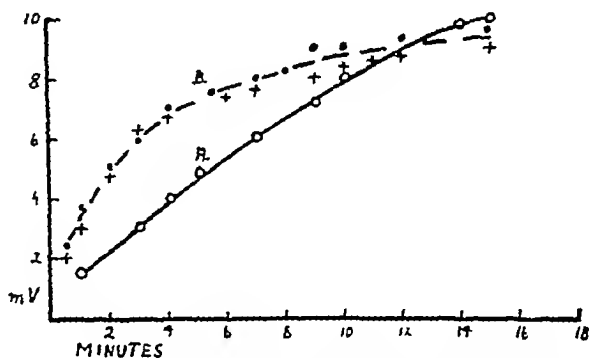


FIG. 4. Depolarization of the whole isolated sartorius. A. After immersing the 'nervous' part into  $10^{-6}$  ACh (When the pelvic end alone is lowered into ACh no potential develops). B. Muscle lowered into 0.22 per cent potassium. Full circles: depolarization at the pelvic end. Crosses: depolarization at the 'nervous' part.

spots on the muscle. Muscles in which a threshold concentration of ACh caused a very small depolarization were sometimes immersed into an ACh solution of  $10^{-3}$  concentration. 10-20 mV could then be found at the nervous part while little (1-2 mV) or no potential was recorded at the pelvic end. The small size or absence of potentials at the pelvic end and the varying size on the nervous part strongly suggests a dependence on endplate foci along the muscle. This was further confirmed by the action of curarine, diminishing or even abolishing these depolarization potentials to all the drugs except KCl. Such an opposing action of curarine to ACh depolarization in muscle has been described by Cowan (15) and the curarine-nicotine antagonism is well-known since Langley's experiments (28).

The effect of eserine in potentiating the local negative potentials set up by ACh was striking in some experiments, but very small or even absent in others. Part of the preparation was lowered into ACh ( $10^{-6}$  or  $10^{-5}$ ) for 10-15 min, then the muscle was washed with Ringer until the potential at the nervous part of the muscle disappeared. After placing the preparation into  $10^{-3}$  eserine-Ringer for  $\frac{1}{2}$  hour the muscle was lowered into an eserine-ACh

similar to the depolarization caused by the 'transmitter action' after nerve stimulation (25, 27) In the present investigation the recording electrodes made contact with the big area and this would certainly diminish the size of the potential recorded from the endplate region

The failure of KCl to set up impulses at the endplate-free regions of muscle is somewhat surprising as it depolarizes the muscle membrane similarly at all points and would be expected to have the same effect as on nerve, for instance on crab nerve (22) Also veratrine resembles potassium in that equally strong after-potentials appeared on the 'nervous' and pelvic part of locally veratrinized muscle, though most of the repetitive after discharges originated from the innervated part (Katz, unpublished observations) The above results could possibly be explained if the rate of depolarization by potassium is actually slower off the endplate, but the difference might not be recorded on account of diffuse leading An additional excitation might occur at the endplate by liberation of ACh by potassium, just as is observed in the superior cervical ganglion (21, 8)

A selective effect of ACh in initiating the discharge of impulses from the endplate could be expected from the experiments of various authors (9, 5, 6, 13) (for an extensive review see Brown, 7) Such an excitability could be well explained by the specific depolarizing action as found in the present experiments This might also apply to ganglion cells which respond to much lower concentrations of ACh than preganglionic or postganglionic fibres (4) Accommodation, however, seems much quicker in muscle than in ganglion cells These latter continue to discharge for many minutes provided the ACh concentration of the perfusion fluid is maintained (4) In muscle only a few bursts of impulses are set up if the endplate region is immersed into a bath of threshold concentration of ACh

The initiation of slow waves by caffeine in frog's brain (28) might also be due to a selective depolarizing action of this drug

It is not possible to determine whether impulses can be set up by application of drugs at endplate-free regions of denervated muscle In some isolated fibre preparations the muscle seemed excitable everywhere, but one cannot exclude the spread of the drug to an endplate near the point of application It also can not be established where the endplates are and how many there are on a denervated muscle fibre preparation The evidence from the whole normal or denervated sartorius supports the view that no impulses are set up at the endplate-free regions Those apparently arising there are probably due to the spread of the drug to some endplates nearby If impulses were set up at the pelvic end of the muscle an appreciable depolarization of the membrane should appear there This could not be observed after ACh, nicotine and caffeine had been applied to the pelvic end of the sartorius The outstanding change in the muscle following denervation seems to be the greatly increased excitability of the endplates This is of interest, as it suggests strongly that spontaneous fibrillation is entirely due to impulses arising at the most excitable part of the muscle, that is at the endplate

As in normal muscle, practically no depolarization occurs at the pelvic end of the whole sartorius if immersed into concentrations of ACh, nicotine or caffeine 10 to 100 times above threshold, while with a just threshold concentration a potential develops at the 'nervous' part of the muscle. A paralytic dose of curarine diminishes the depolarization and also raises the threshold at which locally applied drugs set up impulses. Again, the KCl threshold is not appreciably affected by curarine.

These findings show that the difference in the depolarizing action of drugs on the endplates and the rest of the muscle also persists in chronically denervated muscle.

### DISCUSSION

Chemical depolarization of membranes has been frequently observed. The depolarizing action of potassium on the muscle membrane is well known and a powerful effect of acetylcholine (ACh) in reducing injury potentials has been shown by Cowan (15). When diluted alcohol or veratrine are applied to crab nerve the membrane becomes depolarized and a train of impulses is set up (1, 2). A similar stimulating effect is caused by potassium application (22).

At the endplate region a local potential can be produced by drug application (Fig. 2Ab) and may reach a critical value and then set up either local (Fig. 2Bc) or propagated responses (Fig. 2 and 3). The same sequence of events, as above, can be seen after application of a catelectro-tonic potential, produced by constant current pulses, to isolated muscle fibres (27). Further, subthreshold depolarization and local responses can be set up by the endplate potential in curarized and refractory muscle (20, 25, 26). Thus the excitatory action of drugs is similar to an applied current pulse and to the 'transmitter action' underlying the endplate potential. It seems clear from the present experiments that depolarization of the muscle membrane is caused by certain drugs at the point of application.

The specific excitatory action of ACh, nicotine and caffeine at the endplate is well in line with the selective depolarizing effect at that region. It shows that the endplate is a specialized part in the nerve-muscle system and has a different chemical excitability from nerve or muscle. Distinct membrane properties of the endplate region which may account for this different excitability have not yet been found. Electrically a special excitability, as Lucas's  $\beta$  excitability could not be shown on the whole muscle (33). The fact, however, that the  $\beta$  excitability can be reduced or abolished by curarine is very suggestive that Lucas was stimulating the endplate region, as neither muscle or nerve is appreciably affected by curarine. When depolarization is caused by application of drugs, impulses are set up at the endplate region at a critical threshold level, in agreement with other excitable membranes (23). The height of the depolarization potential was in the present experiments between 20 and 40 per cent of the subsequent spikes. It is possible that at the endplate itself the membrane becomes completely depolarized.



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Du Bois-Reymond (17) and others state that no potential differences exist between two uninjured parts of a muscle (cf for extensive discussion Biedermann, 3) A potential difference is observed only when the muscle fibres are cut, crushed or otherwise injured However, potential differences have been found between different parts of the muscle surface in frog, increasing when the recording electrodes were moved further apart (11) Buchthal and Lindhard (12) have also found that in lizards a potential normally exists between the endplate and the adjacent region of the muscle fibre The endplate was either positive or negative relative to the fibre and sometimes very great potential differences were found (cf 14) In the present experiments these large potential differences could not be found in frog's muscle It is difficult however, to exclude small potential differences with the presently employed fluid electrodes, because potentials confined to the small area of the endplate itself (0.1–0.2 mm) may not be recorded unless a very well defined contact with that area is made

#### SUMMARY

Properties of the endplate and endplate-free regions were investigated in single nerve-muscle fibre preparations of the M adductor longus and in isolated sartorius muscles of Australian frogs (*Hyla aurea*)

1 Acetylcholine, nicotine and caffeine set up impulses by depolarizing the muscle membrane at the endplate region None of the drugs appreciably depolarizes endplate-free parts of muscle, or sets up impulses there

2 Potassium initiates impulses at the endplate region only, but no striking difference could be detected between the depolarizing action at or off the endplate

3 Curarine opposes the depolarization and excitation caused by the drugs, except potassium

4 The sensitivity at the endplates to acetylcholine, nicotine and caffeine in chronically denervated muscles (3–11 weeks) is increased from 1,000 to 100,000 times and is diminished by curarine The selective depolarization of the endplate region and the lack of depolarization at the endplate-free regions pertains also to denervated muscle

5 No appreciable potential differences are found in normal uninjured muscle along the outside of isolated fibres No evidence was obtained of potential gradients around the neuromuscular junction

#### ACKNOWLEDGMENT

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test two-neuron-arc reflex a small filament of the L7 or S1 dorsal root segregated for conditioning stimulation. The size of the selected filament was such that a maximal volley evoked within it would not secure a neuron-arc reflex discharge within the motoneuron pool to be tested. Inhibition of the whole, or some fraction, of the L6 dorsal root was stimulated to secure the 'inhibitory' volley (cf 8).

*The discharge of motoneurons* Between the size of a dorsal root synaptic volley and the size of the discharge evoked from motoneurons

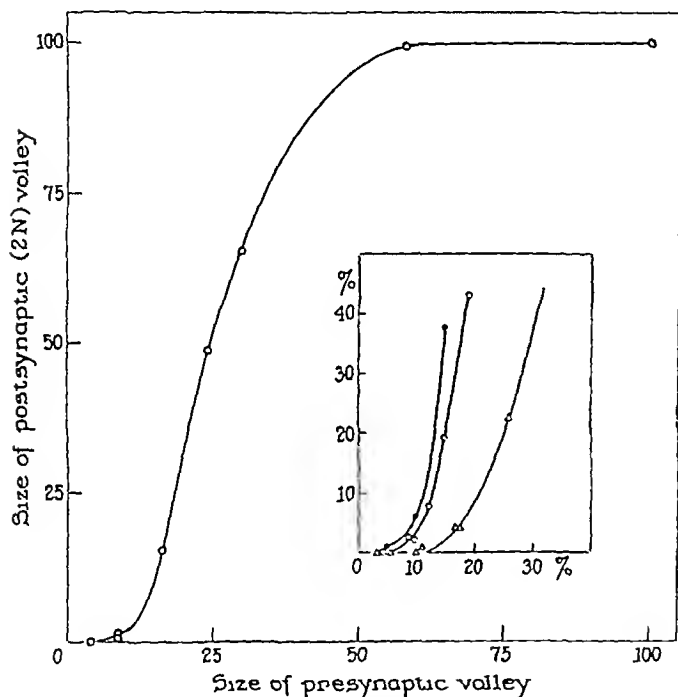


FIG 1 Dorsal ventral root reflex preparation. The size of two-neuron-arc (2N) reflex discharge is plotted against the size of afferent volley evoked by the reflex. Inset: initial portions of several curves from other experiments to illustrate the variation in onset of the reflex discharge from one experiment to another. The curves are constructed from observations such as recorded in Fig 2 description in text.

the direct action of that volley, a definite relation holds. The relation found to follow a sigmoid curve. As may be seen in the experiment plotted in Fig 1, the reflex volley begins to appear only after the dorsal root synaptic potential has reached 7-8 per cent of its maximum. The inset of Fig 1 illustrates the variation encountered from one experiment to another. Once a discharge zone is secured within the segmental pool the size of the reflex postsynaptic volley increases rapidly and apparently linearly with increase in the presynaptic volley until the latter volley is approximately 50 per cent maximal. The reflex volley is then from 90-95 per cent complete. Bearing in mind the relationship between spike height, fiber size and conduction time (5) it is evident that stimulation of considerably less than one half of the fibers contributing to the initial spike potential of the dorsal root secures very nearly the maximum attainable two-neuron-arc postsynaptic volley.

# REFLEX ACTION IN RELATION TO PATTERN AND PERIPHERAL SOURCE OF AFFERENT STIMULATION

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THE SEGMENTAL reflex discharge obtained by single shock stimulation of a dorsal root while recording from the ventral root of the same segment displays a prominent initial peak, which is transmitted through arcs of two neurons, followed by an irregular discharge elevation of some 10 msec duration, all of which is presumed to represent discharges mediated through more complex arcs involving interneurons (7, 9, 11). No concerted effort has been made to relate the type of reflex discharge obtained to the size (*i.e.* the fiber spectrum) of the dorsal root volley employed, although considerable information is at hand concerning the fiber sizes and conduction rates of the various afferent fibers entering into the constitution of the nerves and dorsal roots (3-6, 10, 13). The experiments presented here have as their aim, therefore, the correlation of the central effect of an afferent volley, in terms of reflex discharge, facilitation and inhibition, with the fiber size range and peripheral origin of that afferent volley.

The afferent fibers involved in the present experiments are those which contribute to the first spike elevation in the dorsal root. This elevation is impressed upon the recording system essentially by the A fibers of the dorsal root exclusive of those belonging to the delta sub-group. The action of the delta fibers has not been considered in the present experiments. It should be noted however that the addition of delta fibers to the already active fibers of faster conduction and larger diameter, contributes immeasurably to the occurrence of afterdischarge in the resulting reflexes (14, cf. also 1, p. 565).

By recording directly the volley evoked in a given dorsal root by a single shock, and the results of that sized volley, it is possible to relate the intensity of end action and response to the size of the causal afferent volley. In the accompanying graphs the size of the afferent volleys is plotted along the abscissa, and is expressed in terms of the maximum attainable initial spike potential. The size of the initial (two-neuron-arc) reflex volley (Fig. 1) and the degree of facilitation and inhibition (Fig. 3) of such reflex discharges by appropriate afferent volleys are plotted as ordinates, and are expressed in per cent of the maximum attainable effect. In practice, control observations of the dorsal root spike potential were taken before and after each series of experimental observations. The experimental series in each case was sufficiently long to neutralize the characteristic fluctuations in responsiveness of the 'resting' cord.

The first sacral (S1) segmental reflex was employed for the most part to study the discharge of motoneurons, or as a test reflex response to gauge the effectiveness of subliminal excitation and inhibition. For facilitation of a

the action potential of the multineuron-arc discharges suggests that such discharges become 50 per cent of maximum only after the dorsal root volley is 70-85 per cent maximal. The discharges increase rapidly thereafter. Thus, within the fiber group represented by the initial spike potential of the dorsal root, the lowest threshold members contribute heavily to the two-neuron-arc responses while the higher threshold members account in large measure for the multineuron-arc discharges.

One factor to be considered in evaluating the observations on multi-neuron-arc discharges is the possible effect of the rapidly developing two-neuron-arc discharge. As the two-neuron-arc discharge increases it might be expected to occlude to some extent the ensuing multineuron-arc discharges. However, after the stage represented in Fig. 2E, the factor of occlusion need not be further considered as the two-neuron-arc reflex preempts no more motoneurons to its service, whereas the major intensification of the later discharges occurs only after this stage is reached. Occlusion then is not the important factor. It appears rather that the central connections of the higher threshold fibers are limited to interneurons.

*Facilitation and inhibition* The relationships that have been described show how the motoneurons respond to the end action of the dorsal root fibers. It is possible, by utilizing the variation induced by a measured pre-synaptic volley in the response to a standard test excitation, to measure more directly the end action of presynaptic fibers. Inasmuch as subliminal action is measured, the tests are more subtle than those based purely on discharge response. Since inhibition, in contrast to excitation, must inevitably be assessed in terms of a standard test excitation, the procedure has the advantage of providing a quantitative measure of direct inhibition, and for a comparison between the direct excitatory and direct inhibitory end effects of appropriately selected dorsal root volleys.

In Fig. 3 are plotted the direct excitatory (circles) and direct inhibitory (dots) end effect of appropriate dorsal root volleys upon standard testing two-neuron-arc reflex responses. The intensity of facilitation or inhibition is expressed as a function of the size of the conditioning volley in each case. As in the example provided by reflex discharge, a definite relationship holds between the size of the causal dorsal root volley and the intensity of its end effect (excitatory or inhibitory) in the motor nucleus. The relationship is similar for the two opposed actions. The excitation or inhibition of motoneurons as the case may be, is initially exerted by the lowest threshold fibers in the dorsal root. By the time that the dorsal root volley reaches 50 per cent of maximum, the excitatory or inhibitory action of that volley on the test two-neuron-arc discharge is approximately 90 per cent complete. The virtual identity of the excitatory and inhibitory curves indicates that dorsal root fibers of identical conduction properties are involved in both instances. Moreover it is clear that the lowest threshold fibers in both cases exert most of the end effect.

The simplicity of the two curves suggests that the excitatory and in-

Renshaw (11) has estimated that A fibers conducting at velocities of no less than two-thirds maximal account for the initial reflex discharges. The failure of the reflex volley to increase further as more dorsal root fibers are recruited into the causal volley is due not to a saturation of the motor pool, but is due to the failure of the smaller fibers to reach the motoneurons at all, for it is a commonplace observation that the addition of but a few fibers of the appropriate size range to the stimulated bundle results in a further increase in the postsynaptic volley.

Figure 2 illustrates sample records from a series such as that employed to construct the graph of Fig. 1. The records were obtained from the S1



FIG. 2 Dorsal root-ventral reflex discharges from the first sacral segment. The successive records illustrate the growth of the reflex discharge as the afferent (dorsal root) volley is increased in size. The figures to the right hand of each observation give the relative size of the afferent volley employed for that observation. Time = 5 msec.

ventral root at successively increasing strengths of dorsal root stimulation. The figures to the right of each observation give the amplitude of the dorsal root volley, in per cent of maximum, obtaining for that observation. A two-neuron-arc discharge is first encountered in Fig. 2B, wherein the afferent volley is 10 per cent of maximum. The two-neuron-arc discharge is maximal in 2E, for which observation the dorsal root volley was but 41 per cent of maximum.

Although Fig. 2 shows, with reference to two-neuron-arc discharges, the type of observation employed in constructing the curve of Fig. 1, it is presented primarily to illustrate the relation between the size of a dorsal root volley and the amount of motoneuron discharge secured through the more complex segmental reflex arcs. Some discharge pertaining to the more complex arcs is apparently realized as soon as a two-neuron-arc discharge is discernible, but intense development of the multineuron-arc discharge occurs only as the dorsal root volley increases beyond 50 per cent of its maximal size towards maximality. Estimates based on the area enclosed by

established. It is unlikely therefore that any significant number of neurons are close to or at threshold in the 'resting' pool, for if there were first afferent impulses to enter the pool should secure a postsynaptic charge. The resulting discharge curve would then approximate in form of the facilitation curve.

The diagram of Fig. 4, dealing as it does with synchronous afferent excitation and two-neuron-arc discharges does not contain a time parameter for time is fixed and intensity only need be considered. When, how excitation occurs within the motor nucleus as the result of a diffuse infl

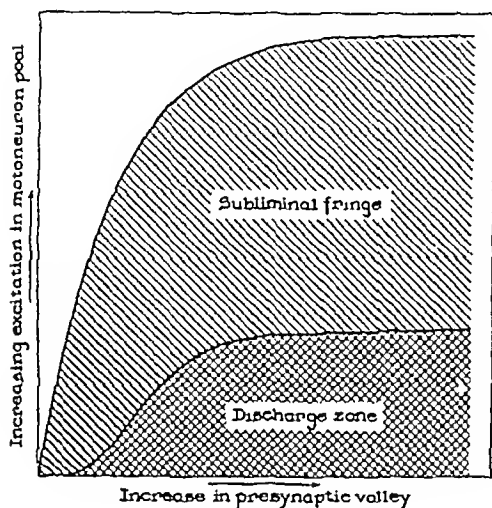


FIG. 4. A diagram based on Fig. 1 and 3, to illustrate the relation between subliminal fringe and discharge zone in a motoneuron pool activated by dorsal root volleys.

ment, aided unquestionably also by the development of subnormals among the motoneurons. In short the motoneuron discharge should be considerably synchronized relative to the causal synaptic barrage. This is precisely what does occur, for instance, when lumbar or sacral motoneurons are excited by activity transmitted through the long spinal reflex system.

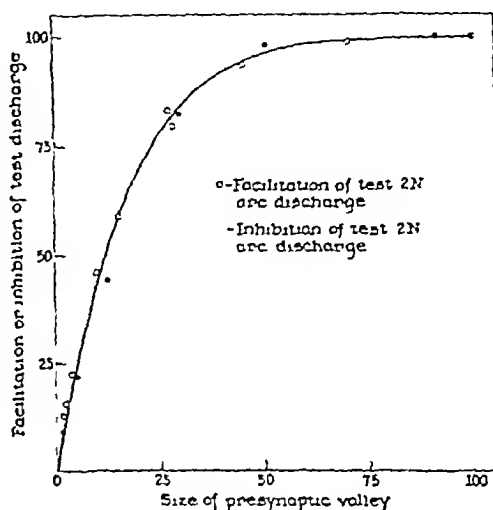
*Peripheral origin of afferent fibers contributing to two-neuron-arc, and later reflex discharges.* From a study of Fig. 1, 2, and 3 it is seen that the threshold dorsal root fibers mediate direct effects to the motoneurons, whereas the higher threshold fibers act indirectly through internuncial relays. Since the low threshold fibers are those of largest diameter, and those which conduct impulses at the greatest velocities, it follows that the large, rapidly conducting fibers of the dorsal root are responsible for direct action on motoneurons.

The most rapidly conducting afferent fibers have a conduction velocity

impulses, rising and falling in intensity over a number of milliseconds as in the instance presented by spinal reflex excitation (9), changes plotted in Fig. 4 as a function of intensity of presynaptic excitation, will take place with intensity as an added parameter. In effect, the events will progress initially to the right along the abscissa as the pulse bombardment increases in intensity, and subsequently to the left as the impulse bombardment wanes. On this schema the motoneuron discharge realized as the result of a diffuse waxing and waning excitation should appear only after a considerable nuclear delay occasioned by the degree of excitation necessary to secure a discharge zone, and should cease considerably before the presynaptic bombardment

hibitory end effects are separate and not intermingled under the anatomical and temporal restrictions employed in these experiments. However, the very identity of these curves makes it conceivable that the two end effects as measured in Fig 3 could be intermingled and yet appear as pure processes. For instance, if the two opposed processes were intermingled and were to maintain a constant proportionality at all strengths of the causal dorsal root volley, then the resulting experimental curve, representing the difference, would indicate only the more powerful, and would differ in no way from

FIG 3 A comparison between the development of direct excitation and direct inhibition of motoneurons. Circles the course of facilitation of a standard test two-neuron-arc reflex plotted against the size of the conditioning dorsal root volley. Each point represents the difference between the facilitated response and the control response expressed in per cent of the difference obtained by use of the maximal conditioning volley. Dots the course of direct inhibition similarly plotted, but from another experiment. Each point represents the difference between the control response and the inhibited response expressed in per cent of the inhibition obtained by use of the maximal conditioning volley. Further details in text.



the curves to be expected from exquisitely segregated excitatory and inhibitory processes.

Simple and superimposable curves, such as those illustrated in Fig 3, would not be obtained if excitatory and inhibitory action differed in any way related to the fiber population occupied by the causal afferent volleys. Therefore, the fibers which mediate direct excitation and direct inhibition to the motoneurons are indistinguishable on a functional basis. There is, in consequence, no reason to suppose that a special group of fibers is employed in each case.

*Relation between subliminal fringe and discharge zone in simplest reflex system.* Figure 4 represents diagrammatically the relation between the subliminal fringe and the discharge zone within a motoneuron pool upon the occasion of direct excitation through dorsal root collaterals. The diagram is based on the experiments described in connection with Fig 1 and 3.

The initial upward concavity, present in the discharge curve (cf Fig 1) but absent in the excitation curve (cf Fig 3) undoubtedly reflects the need for summation within the motor nucleus before discharge may occur. Accordingly in the 'resting' preparation a synchronous volley of considerable dimensions must reach the motor nucleus in order that a firing zone may be



and a single synaptic relay. There is a small delayed discharge appearing with a total latency approaching 6 msec. In Fig. 4B is recorded the reflex discharge obtained in the ventral root by stimulation of the sural nerve. This record the earliest reflex discharge appears with a total latency of about 5 msec. Since, in its afferent course the sural nerve-ventral root reflex must travel in fibers of slower conduction than does the gastrocnemius nerve-ventral root reflex, some of the added latency may be accommodated as added conduction time. The time accounted for by differential conduction rate would be less than 0.5 msec, which means that the sural nerve-ventral root reflex has the longer central latency by about 2.0 msec. Furthermore, the sural nerve-ventral root reflex discharge is irregular and has a duration of about 10 msec, which features are characteristic of the later portions of the familiar dorsal root-ventral root reflex. Thus a segregation of afferent fibers according to their origin in muscle or in the skin results in a high degree of segregation of the two-neuron-arc and later reflex discharges. The two-neuron-arc connections are placed at the service of the afferent influx from muscle, the afferent impulses from cutaneous areas are routed through the more complex and diffuse internuncial systems.

In consideration of the identity of afferent fiber range mediating excitation and inhibition directly to the motoneurons (Fig. 3), it follows that the dorsal root fibers mediating direct inhibition to the motoneurons have their origin within the muscle afferent system.

### SUMMARY

Two-neuron-arc reflex discharges in the dorsal root-ventral root reflex are secured by stimulation of the lowest threshold fibers of the dorsal root. Stimulation of the higher threshold fibers is primarily responsible for the multineuron-arc reflex discharges.

The fibers mediating direct excitation and direct inhibition to the motoneurons are functionally indistinguishable in the dorsal root.

Afferent fibers of the size range having direct connection with the motoneurons are found in significant numbers only in muscle nerves. The group of afferent fibers which accounts heavily for connections to interneurons is prominent in cutaneous nerves, but poorly represented among the muscle afferent fibers (2-5, 10, 13). Utilizing this information it is possible to effect a high degree of separation of the two-neuron-arc and multineuron-arc reflex discharges by employing a muscle nerve and a cutaneous nerve respectively for afferent stimulation.

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of approximately 115–120 M/sec. Employing the factor of six relating conduction rate to fiber size (6), the largest afferent fibers would be approximately  $20\mu$  in diameter, which is in full agreement with the histological observations of Sherrington (13). The present observations and those of Renshaw (11) are in agreement that the upper third of the fiber spectrum accounts substantially for the two-neuron-arc afferent fibers. This would place the bulk of the afferent fibers establishing direct connection with motoneurons in the range above  $12\mu$ – $13\mu$  in diameter.

It is well known that cutaneous nerves contain but few fibers greater than  $12\mu$  in diameter (2, 3, 5), so it is unlikely that the afferent fibers in cutaneous nerves form direct connections with the motoneurons, though there can be no doubt that these fibers contribute heavily to the multineuron-arc reflexes. On the other hand the conspicuous pile in the fiber spectrum of 'demotored' muscle nerves, i.e. motor nerves from which the motor fibers have been degenerated by ventral root section, lies in the diameter range above approximately  $12\mu$  (2, 10, 13), whereas the number of fibers between  $12\mu$  and  $6\mu$  is scant. It follows from these several observations that the two-neuron-arc-reflex discharge should result from stimulation of the afferent fibers from muscles, but not from the stimulation of cutaneous nerves, while stimulation of the cutaneous nerves should result in a profound reflex discharge of greater central latency. This conclusion has been put to test in the following manner and established as correct. The sural nerve and the nerves to the gastrocnemius muscle are utilized to provide a cutaneous afferent volley and a muscle afferent volley respectively. The L7 and S1 ventral roots are severed to provide a means of recording the reflex discharges, and incidentally to avoid the untoward effects of antidromic volleys sweeping from the gastrocnemius nerve into the motoneurons (cf 12).

Figure 5A presents the reflex discharge recorded from S1 motoneurons and evoked by an afferent volley in the nerves to the gastrocnemius muscle. The feature in this record is the two-neuron-arc discharge appearing with a total latency of 2.4 msec, which is only sufficient to account for conduction

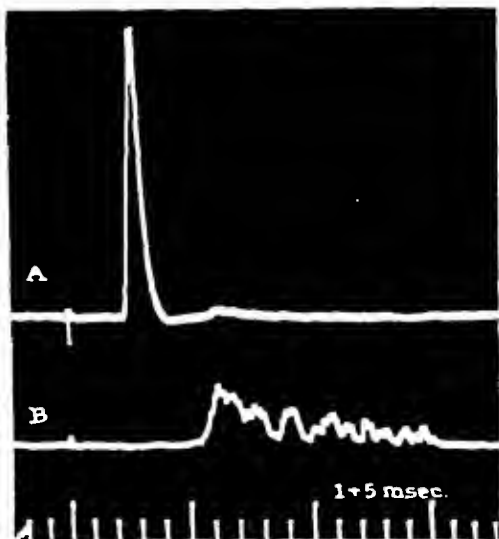


FIG 5 Reflex discharges recorded in a ventral root and resulting from maximal afferent volleys in (A) the nerves to the gastrocnemius muscle, and (B) the sural nerve. The total conduction distance for the two reflexes, and the amplification employed are comparable. Further description in text.



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lobe As will be shown presently, a portion of this second part stands in functional relation to the acoustic area while the rest does not We shall therefore divide the temporal lobe into an acoustic and a temporal sector The precise location of the boundary between them presents one of the problems of this investigation

#### METHOD

The functional organization of the temporal lobe was investigated by the study of changes of its electrical activity in response to various stimuli The experiments were per-

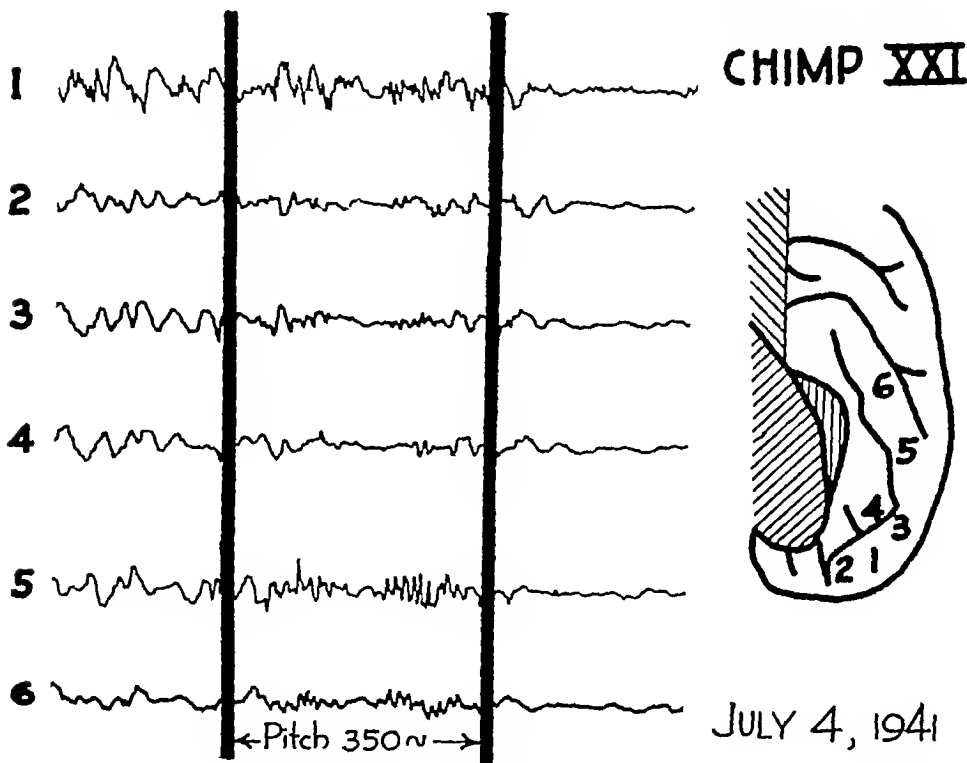


FIG 1 Response to acoustic stimulation of supratemporal plane of chimpanzee Sketch on right shows supratemporal plane with position of pickup electrodes Records on left show cortical responses Numbers 1 to 6 correspond to numbers of electrodes in unipolar pickup

formed upon several monkeys (*Macaca mulatta*) and two chimpanzees, in each case under moderate Dial\* anaesthesia Practically the whole of one hemisphere was exposed by wide opening of the skull and dura mater In order to reach the inferior aspect of the temporal lobe, the animal was securely fastened with its belly on a board This board was then inverted so that the animal hung supine After cutting the anastomotic vein of Labbé the brain had sagged out enough in about one hour to give access to its basis In order to expose the supratemporal plane the animal was righted again and the parietal operculum was

\* 0.45 cc gr per kg,  $\frac{1}{2}$  intraperitoneal,  $\frac{1}{2}$  intramuscular We wish to thank Ciba for putting the Dial at our disposal

# FUNCTIONAL ORGANIZATION OF TEMPORAL LOBE OF MONKEY (*MACACA MULATTA*) AND CHIMPANZEE (*PAN SATYRUS*)

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## INTRODUCTION

EXPERIMENTS on the sensory and adjacent cortex of the monkey and chimpanzee (2, 12, 3) frequently necessitated controls by placing electrodes or applying strychnine to other parts of the cortex. The incidental information so acquired gave valuable clues for further experimentation. The functional organization of the occipital lobe, centering around the primary visual cortex, has already been reported (6). It was hoped that, by concentrating attention upon the temporal lobe, it might similarly be possible to make out the functional organization centering around the primary auditory cortex. For that reason the present experiments were undertaken.

The temporal lobe, as that term is used in anatomy, is completely covered by isocortex. It does not include the pyriform area and the hippocampal formations—the cornu ammonis proper and the entorhinal and adjacent areas. On cytoarchitectural grounds Campbell (10) divided this part of the brain in man, chimpanzee and orang into an audiosensory, an audiotpsychic and a common temporal area. Brodmann (7, 8) sub-divided in man the common temporal area into areas 22, 21, 20 and 38, in the cercopithecus into 22, 21 and 20. Mauss (18, 19) recognized a temporo-polar area in the orang and gibbon but not in the cercopithecus. V. Economo and Koskinas (14) divided the temporal lobe of man much in the same way as Brodmann. Brodmann and Mauss failed to mark an audiosensory or audiotpsychic area in the monkey. In man, Brodmann (7) described an area 42 as the audiosensory and an area 41 as the audiotpsychic area. In a later contribution (8) he stated that the "regio supratemporalis" could be divided into four areas 22, 41, 42, 52. Mauss recognized two areas in the orang, which he called 40 and 38, but only one in the gibbon, which he labeled 40.

The study of the thalamocortical relations in the macaque and chimpanzee (25-28) suggests a division of the temporal lobe into two major parts: (1) an area receiving radiations from the medial geniculate body, and (2) a much larger part devoid of thalamic radiations. The first of these is the audiosensory area and covers no more than a few square centimeters on the supratemporal plane. The second of these comprises the rest of the temporal

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It proved possible to map out a tonotopic localization in the comparatively large brain of the chimpanzee. As Fig 2 shows, low pitches are picked up chiefly in the antero-lateral portion, and high pitches in the postero-medial portion of the acoustic area. The cortical surfaces from which responses to different stimuli were picked up overlap appreciably. Whether this is to be explained by the physical conditions of the pick-up, or by the

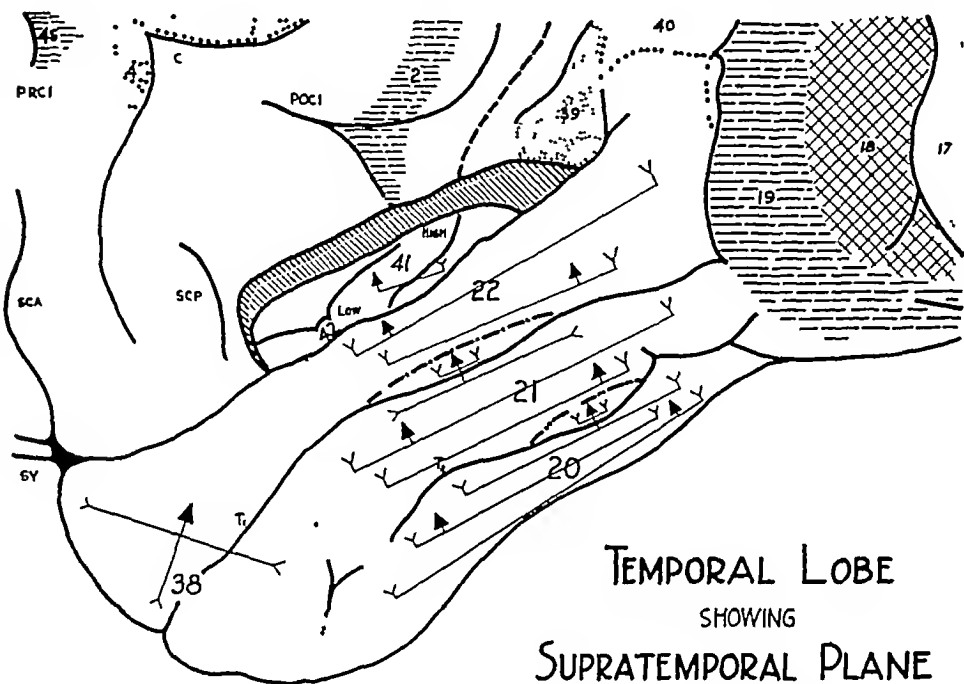


FIG 3 "Firing" diagram of temporal lobe of chimpanzee. The supratemporal plane is exposed by resection of parietal operculum. c sulcus centralis. poci sulcus postcentralis inferior. prci precentralis inferior. sca sulcus centralis anterior. scp sulcus centralis posterior. sy sylvian fissure. T<sub>1</sub> sulcus temporalis superior. T<sub>2</sub> sulcus temporalis medius. The numbers correspond to Brodmann's areas. For details see text.

unavoidable background noise, or by overlapping of the fibers of the acoustic radiation, cannot be decided.

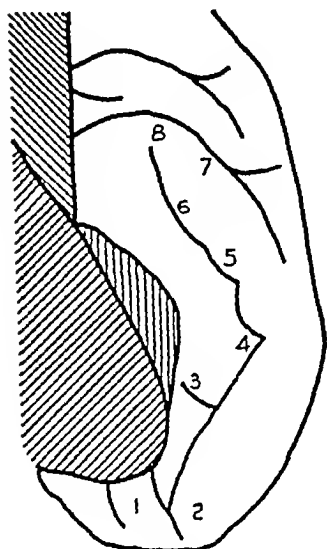
When strychnine is applied locally to the primary auditory cortex, as defined by the previous experiments, spikes are propagated into area 22 on the lateral side of the temporal lobe, but nowhere else. Conversely, strychninization of area 22 causes spikes to appear in the primary auditory cortex as well as elsewhere in 22.

2 *The temporal sector.* The rest of the temporal lobe can be subdivided in both macaque and chimpanzee into three areas. Strychninization of each area "fires" all of that area but does not "fire" any point in any other area, as Fig 3 is intended to show. The areas so mapped out correspond roughly

carefully resected subpially by suction, so as to preserve the temporal pia mater and blood supply. A brain so prepared will exhibit essentially normal electrical activity for as much as 72 hours. Silver electrodes were placed at all points to be investigated. In most animals 36 electrodes were used and records made synchronously from 6 of them at a time. The technique has been described in detail in previous publications (6, 12).

## RESULTS

1 *Acoustic sector* In the intact brain electrical activity can be stopped or greatly reduced by any jolt or sudden sound sufficiently intense to startle the beast. Properly speaking, this abatement of activity is not an auditory



ELECTRODES	PITCH						
	100	200	400	800	1600	3200	6400
1—2	o	o	o	o	o	o	o
3—4	?	+	o	o	o	o	o
5 →	+	+	o	o	o	o	o
6 →	+	+	+	+	o	o	o
7 →	o	+	+	+	+	o	o
8 →	o	o	+	+	+	+	o

## TONOTOPIC ORGANIZATION SUPRATEMPORAL PLANE ~ CHIMPANZEE XXI

FIG. 2. Tonotopic organization of the supratemporal plane of chimpanzee. At right, responses of electrodes to various pitches; at left, position of electrodes.

affair, for jolting is as effective as sound, and it affects all parts of the cortex approximately equally and approximately synchronously. A different reaction, however, is observed in the posterior part of the supratemporal plane,—that is to say, in that part which is the primary auditory cortex. From here an increase in electrical activity upon the beginning and the end of an acoustic stimulus can be observed. Figure 1 shows a typical response to a frequency of 350 cycles per sec. It shows the increased activity at the onset followed by an abeyance of activity which is part and parcel of the generalized diminution due to startle. This reaction can only be observed in the acoustic area. Occasionally, however, one can just see, synchronously with the abeyance in the acoustic area, a small disturbance appearing in the immediately adjacent cortex of the supratemporal plane. This phenomenon has never been observed on the lateral side of the temporal lobe.



It proved possible to map out a tonotopic localization in the comparatively large brain of the chimpanzee. As Fig 2 shows, low pitches are picked up chiefly in the antero-lateral portion, and high pitches in the postero-medial portion of the acoustic area. The cortical surfaces from which responses to different stimuli were picked up overlap appreciably. Whether this is to be explained by the physical conditions of the pick-up, or by the

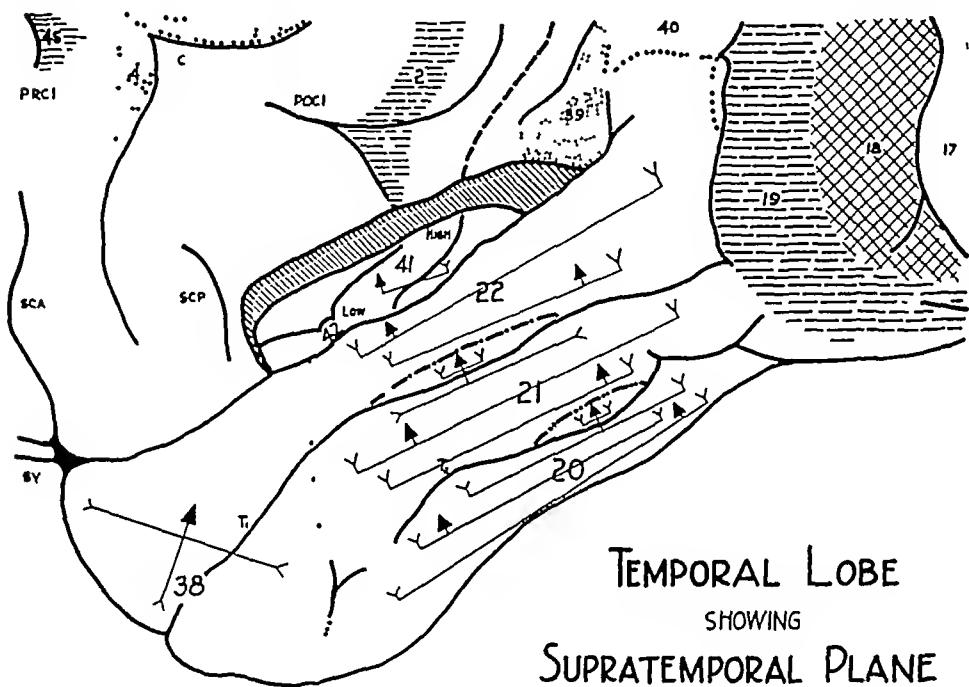


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to Brodmann's areas 21, 20 and 38, as shown in the human brain. However, the first and second temporal sulci are no more than approximate landmarks, for in both monkey and chimpanzee areas 20 and 21 protrude slightly over the dorsal lip of the second and first temporal sulcus, respectively. Obviously, in both cases the area ventrally adjacent to the sulcus covers both of its walls.

The problem finally arose whether commissural connections between the two temporal lobes existed. In the intact animal "cross-firing" was observed only from the second temporal convolution—i.e., from area 21, to the homologous loci on the other hemisphere. The appearance of strychnine spikes in the contralateral hemisphere was not abolished by cutting the corpus callosum, and it is concluded that the commissural fibers between the two temporal lobes take their course through the anterior commissure (3, 20).

As has been shown elsewhere (2, 6), area 20 receives afferent impulses travelling through the inferior longitudinal fasciculus from the parastriate area 18, and area 38 receives impulses through the uncinate fasciculus from the area orbitalis agranularis, the homologon of Brodmann's area 47 and v. Economo and Koskinas' area FFa in the human brain.

### DISCUSSION

The work reported in this paper confirms the conception of Campbell and Walker that the temporal lobe can be divided into two sectors, an acoustic or supratemporal and a temporal sector. Our work also makes it clear that, contrary to Brodmann's statement, there is in the brain of the monkey and chimpanzee an area homologous to what Brodmann called 42 in the human brain. A koniocortex on the supratemporal plane was indeed described by Walker (25) for the macaque, and also mentioned at about the same time by v. Bonin (5) for the cebus. The minute myeloarchitectural studies by Beck (4) on the chimpanzee are hard to evaluate. Examinations of several brains of macaques and chimpanzees have shown that both primates possess an area of koniocortex on the supratemporal plane and that this koniocortex is developed to a different degree in different parts of that area, just as it was demonstrated for man in the detailed study by v. Economo and Horn (15).

The tonotopic organization found in the macaque and chimpanzee confirms Pfeifer's (22) surmise concerning the human brain to which he was led by a critical survey of clinical observations and by his study of the acoustic radiation. It is also in agreement with Walker's (25, 27) studies on the projection of the medial geniculate body on the cortex in the macaque and in the chimpanzee.

The theory advanced many years ago by Polyak (23) on the basis of careful anatomical studies has been confirmed and implemented by modern physiological research. It has been established that there is a tonotopic organization in the cochlea (11), and Walzl and Woolsey (29) have demonstrated in the cat that there is a topological representation of the cochlea

in the primary acoustic area of the cortex After Ades and Felder (1) had defined the acoustic area of the monkey by click stimulation of that animal, Licklider and Kryter (17) found a tonotopic organization within that area, the lower frequencies activating the antero-lateral part of that region, the higher ones the postero-medial one Our own work confirms Ades and Felder's observations on the monkey, and extends Licklider and Kryter's findings to the chimpanzee Lashley's (16) findings in the rat would indicate that a tonotopic organization is of relatively recent phylogenetic origin

The relation between auditory cortex and area 22 is similar to that between striate and parastriate area, which was reported on by v Bonin, Garol and McCulloch (6)—with this difference, however, that secondary disturbances due to acoustic stimulation were never observed within area 22 They were observed, however, in the immediate vicinity of the auditosensory area—*i e*, in the area temporalis magnocellularis, the homologue of Brodmann's area 42 and v Economo's area TB in the human brain The acoustic sector can therefore be subdivided into three areas, known in the human brain as 41, 42 and 22, or TC, TB and TA respectively

In the temporal sector, area 38 could be identified by physiological methods It was indicated by Brodmann (7, 8) in the human brain as covering the temporal pole, but it was not given on his map of the monkey It was found, however, by v Bonin (5) in the cebus Histological controls showed that the cortex covering the temporal pole in both macaque and chimpanzee differs from that covering the rest of the temporal pole in precisely the same manner in which it differs in man The inner granular layer is much attenuated, both third and fifth layers contain mostly smaller cells, and the cell density is lower than elsewhere in the temporal lobe It is possible to differentiate the temporo-polar area histologically as well as functionally in the brains of all gyrencephalic primates

Our conclusion that the commissural fibers between the two temporal lobes take their course through the anterior commissure confirms what Rundles and Papez (24) and Bucy and Kluver (9) have stated on the basis of anatomical evidence No attempt has been made, however, to cut the anterior commissure and leave the corpus callosum intact It can not be asserted, therefore, that *all* commissural fibers between the two temporal lobes take their course through the anterior commissure

### CONCLUSIONS

The temporal lobes of macaque and chimpanzee show the same functional organization The lobe may be divided into two sectors (1) an acoustic sector, consisting of the primary acoustic or auditosensory area 41, a small area 42 surrounding it, and area 22, mutually spiking each other, and (2) a temporal sector, comprising areas 21, 20 and 38, each of which fires only locally In the primary acoustic area of the chimpanzee tones of low frequency arrive in its antero-lateral part, tones of high frequency in its postero-medial part Within the temporal sector the existence of a temporo-

polar area has been demonstrated in the macaque Commissural connections between the two temporal sectors are restricted to area 21 and take their course probably through the anterior commissure

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turbance of the ipsilateral area 18 to the contralateral. The time of this relayed spike is such that it follows the direct spike by more than an entire spike duration—that is, by more than 50 msec (Fig 1). This is considered worth reporting because of the strength of the commissural connections be-

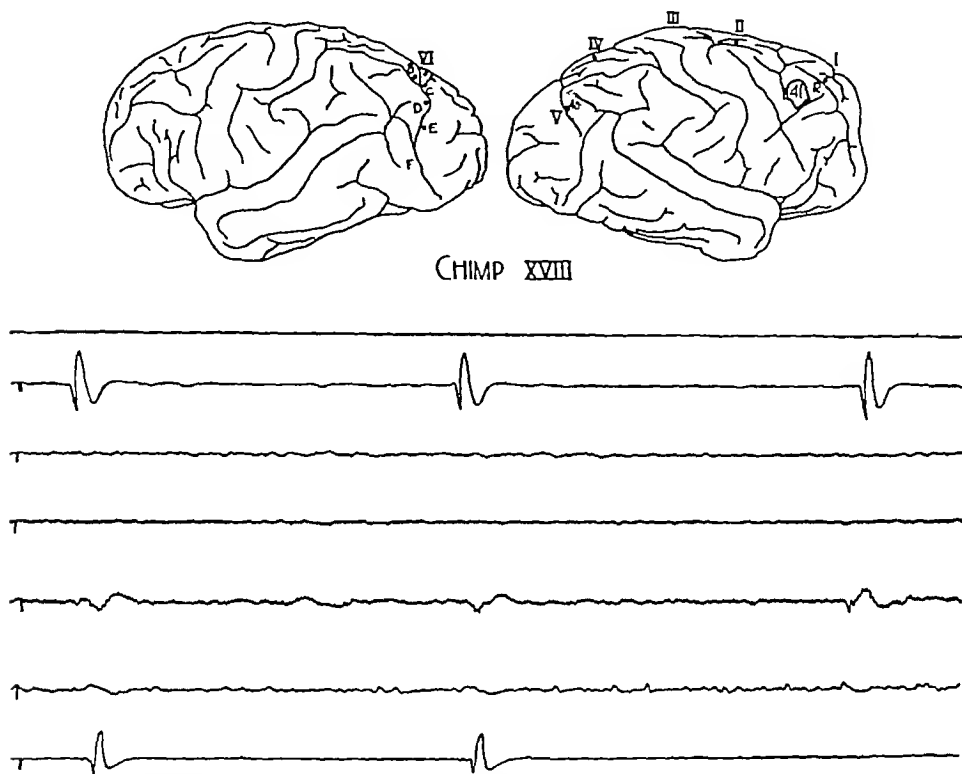


FIG 1 CHIMP XVIII Strychninization 41. The outline sketch of the brain shows location of strychnine 41, location of previous strychnine 40, and location of sixth row of electrodes on the contralateral hemisphere. The spikes from strychnine 40 are now set off by the small spikes from strychnine 41. The top tracing is from the rover (*R*) at a gain of 8 H. The next four tracings are from cortical leads, *ab*, *bc*, *cd*, and *de* of row six on the contralateral hemisphere, at a gain of 4 H. (some 60 cycle disturbance in *cd* contralateral lead). The last tracing is from electrode *V* of the ipsilateral hemisphere, at a gain of 8 H, and shows small direct spikes from strychnine 41 followed by large spikes which they provoke from the partial residual strychninization of 40. At the right hand is a spike from strychnine 41 transmitted to contralateral *cd*, at the center a spike from strychnine 40 which fires contralateral *cd*, at the left hand both spikes fire into *cd*. Preceding each spike at *V* from strychnine 40 is a smaller disturbance transmitted from the strychnine 41. Paper speed 6 cm/sec, tracing reduced 59 per cent.

tween the area 18 (1) Strychninization anywhere in area 18, whether on the medial, lateral or inferior surface of the hemisphere, results in strychnine spikes propagated to some part of the inferior temporal convolution (area 20) (3) In general, those from the more inferior portion of area 18 are propagated further toward the tip of the temporal lobe. On several occasions

# LONG ASSOCIATION FIBERS IN CEREBRAL HEMI-SPHERES OF MONKEY AND CHIMPANZEE\*

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DURING experiments on the functional organization of the cerebral cortex in the monkey and chimpanzee, as well as during studies on its projection systems, attempts were constantly made to prove physiologically the existence of those long association tracts joining remote regions of the cerebral hemispheres which have been described in the human brain. Several have been found but, as they belong in no case to any one of the anatomical or functional subdivisions of the hemisphere, it was thought best to report them in a separate article. The long callosal fibers have already been described (1) and will not be herein mentioned, except for the interhemispheric connections of area 18.

## METHOD

The method of physiological neuronography has been explained in previous articles (2). All experiments were performed under full Dial anaesthesia, with the brain exceptionally widely exposed and with the pia mater and cerebral blood supply and drainage as nearly intact as possible. Electrodes were placed, sometimes at one site and sometimes at another, so that practically all parts, excepting the most medial portion of the inferior surface behind the temporal lobe, had been examined by the end of the experiments. The same procedure was followed with respect to strychninization. This was in each case performed by application of a few square millimeters of filter paper moistened with a saturated solution of strychnine sulphate. The activity was recorded before and after the strychninization. Photographs were made and the positions of electrodes and strychnine recorded on these. After sacrificing the animal the brain was peeled, rephotographed and studied to determine as precisely as possible the sites of electrodes and strychnine and thus to prepare the composite diagrams presented below.

## RESULTS

The experiments disclosed the existence of three long fiber tracts, originating and terminating on the convex surface of the hemisphere. (1) in the chimpanzee, strychninization of the posterior margin of band I, which by stimulation was proved to be the eye field (area 8 of Brodmann) produces well-defined strychnine spikes propagated to area 18 of the same hemisphere, and also to the corresponding area of the opposite hemisphere [see (5), Fig. 2]. By partial strychninization of the ipsilateral area 18 during this strychninization of band I, it was possible to obtain a relaying of the dis-

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licated Finally, no other tracts were found connecting equally remote portions of the hemisphere \*

### DISCUSSION

The method of local application of strychnine and recording action potentials has been shown to disclose not only the existence but also the direction of the fibers between the gray masses of the brain, for strychnine acts only where axonal terminations contact cell bodies and causes disturbances propagated in the normal direction, not antidromically Thus it was reasonable to expect that its application to the problem of the direction of the long associational tracts of the cortex would help to show in what areas these systems originated and terminated Burdach, (1819-1826), Arnold (1838) and others, by dissection of the human brain, discovered four main associational bundles

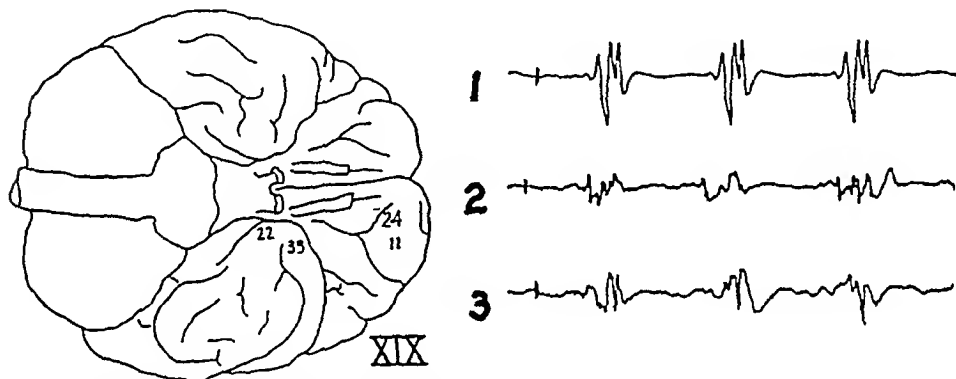


FIG 3 CHIMP XIX Strychninization 24 Firing (11) at site of strychninization and (22) and (33) at tip of temporal lobe Bipolar recording Sketch of the ventral surface of the brain shows area strychninized and location of electrodes

but, because of obvious deficiencies of the method, had not been able to determine accurately their origins or terminations Subsequent anatomists have usually been content with schematic representations, one of which, ascribed by Tilney and Riley to Meynert, is given below (Fig 4) It was a matter of surprise that each of the three associational bundles herein reported was found to be unidirectional in its transmission, and to arise and to terminate in a functionally and cytoarchitectonically unique area of the cerebral cortex, as indicated in Fig 4

In addition to the long longitudinal fiber systems others, running in a transverse direction, have been described in the human cerebral hemisphere Those described within the occipital cortex—vertical occipital fasciculus of Wernicke, stratum proprium cunei of Sachs, transverse occipital fasciculus of the lingual lobule of Vialet—are evidently, as was shown by v Bonin,

\* In recent experiments on the medial surface of the hemisphere exceptions to this statement have been found

this disturbance was found to extend just onto the central portion of the upper lip of the second temporal sulcus, but at no time could these disturbances be traced to the extreme tip of the temporal lobe, either in the monkey or in the chimpanzee Figure 2 exemplifies these findings (iii) Strychninization of the area orbitalis agranularis (area 47 of Brodmann or

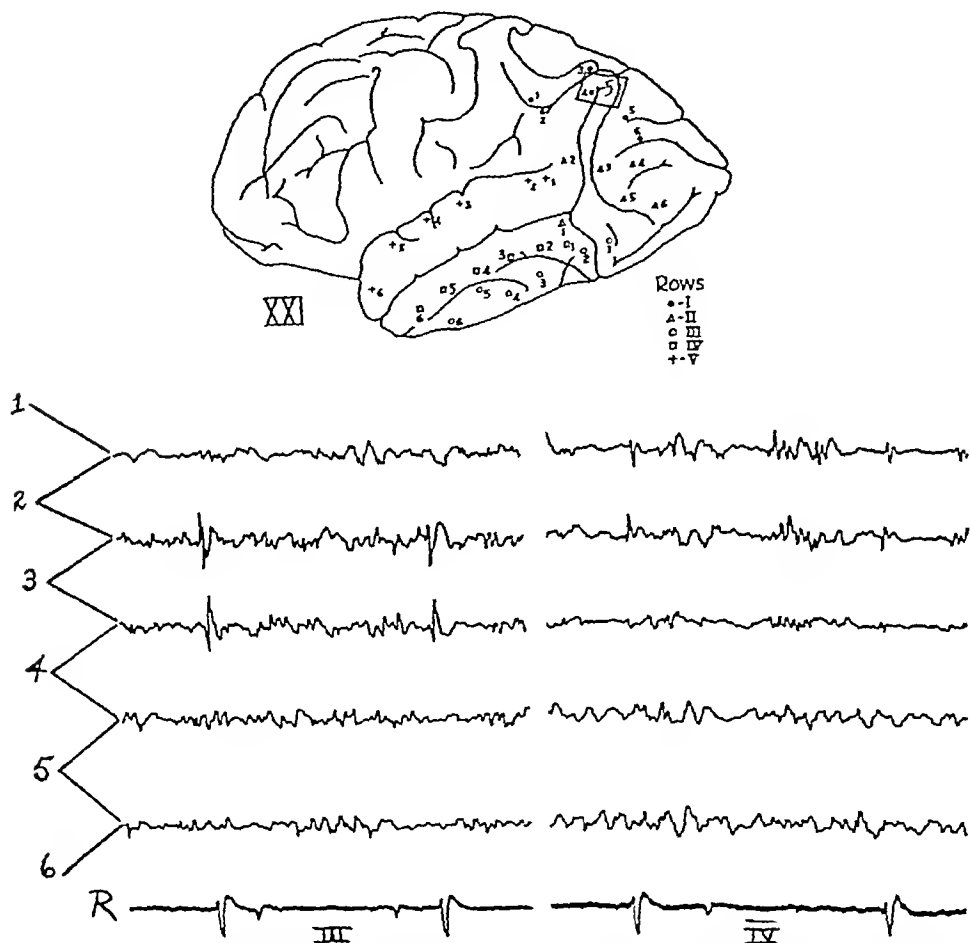


FIG 2 CHIMP XXI Strychninization 5 Left hemisphere Diagram shows localisation of strychnine 5 and position of electrodes The tracings are of rows III and IV Firing in 3 of row IV and in 1, 2 and 3 (feebly) of row III Rover at the bottom

FFA of v Economo in the human brain) produces well-defined strychnine spikes propagated to the anterior portion of the temporal lobe—that is, to what Brodmann calls area 38 in his map of the human cortex Figure 3 exemplifies this

The negative findings are equally important First, at no time were any strychnine spikes propagated in the reverse direction between the areas in-

human cerebral cortex have been disclosed. From area 8, the frontal suppressor area, there arises a tract leading to area 18, the parastriate area, this is probably part of the superior longitudinal fasciculus of Burdach. From area 18 there arises a tract leading to area 20, on the inferior temporal convolution, it is usually called the fasciculus longitudinalis inferior, and may comprise also what anatomically has been called the vertical occipital fasciculus of Wernicke. There is a tract arising from the area orbitalis agranularis (called area 47 in the human brain) passing to the tip of the temporal lobe (called area 38 in the human brain), it is ordinarily called the fasciculus uncinatus. Each of these pathways normally conducts in one direction only.

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Garol and McCulloch (4), fibers connecting various parts of the parastriate area. In general these are not as distinct bundles as are the longitudinal fasciculi. It might be pointed out here that we have found abundant evidence of such long transverse fibers in the firing of entire vertical bands on the convexity of the sensory cortex of the chimpanzee, but rarely of a definite fasciculus arising in one cytoarchitectonic area and terminating in another remote area. At least one such exists, however, in the parietal area of the chimpanzee. It extends from just above the interparietal sulcus to an area just above the posterior part of the Sylvian fissure.

Special emphasis should be laid on the time relations, for the conduction in each of these associational bundles occurs with axonal velocities. In fact, it requires high paper-speed to distinguish on an inkwriter record which

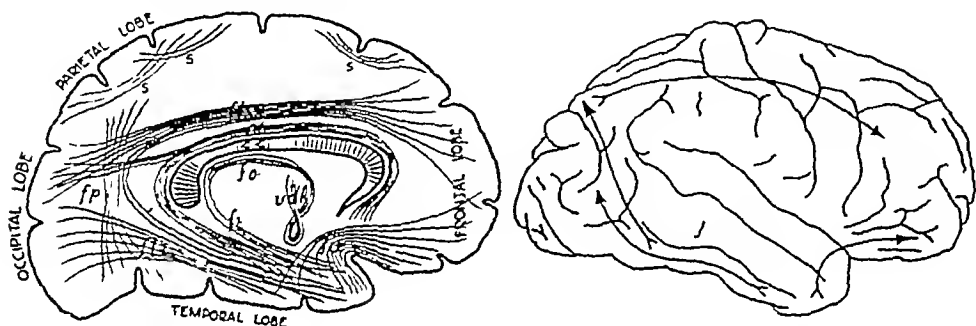


FIG 4 *Left* diagram of long fiber tracts in human brain according to Meynert. Seen from medial surface. *Right* diagram of longest fiber tracts in chimpanzee brain as determined by strychninization and electrical recording.

strychnine spike occurs first—i.e., the one in the propagating area or the one in the recipient area. The contrast between the rapidity of these disturbances and those which are relayed—i.e., are post-synaptic—is brought out by the experiment in which the strychnine spike was conducted the long distance from area 8—i.e., band I—to both ipsi- and contralateral areas 18 at high velocity, whereas the relayed spike crossing from one area 18 to another, after partial strychninization of one of these areas, is delayed so long that the last traces of the axonal termination spike has subsided—in a matter of more than 50 msec (Fig 1).

Consideration of the functional organization of the primate cortex as a whole might suggest the existence of a pathway from the temporal lobe to remote regions of the cortex, but the present experiments have failed to reveal any such structure.

#### SUMMARY

By applying strychnine locally to the cerebral cortex of the monkey and chimpanzee, and recording the electrical activity, the origin and termination of homologues of three of the well-defined long association bundles of the

All three tissues were markedly affected by oxygen tension in the range below 21 per cent. The difference between 100 and 21 per cent was significant for the quantity of data obtained only in cortex. When the oxygen uptake was expressed as a percentage of the value for 100 per cent oxygen (Fig 2), there was no striking difference between the three tissues in the influence of

*Table 1 Lactic acid output during the preliminary period of gassing and equilibration*

Exp	Oxygen mixture	Lactic acid output			Manometric equivalent*
	Vol per cent	(1) Total	(2) Blank	(3) Net for 2 hr	$Q_G$ (4) (4) = (3)/8
mg /gram tissue					mm <sup>3</sup> CO <sub>2</sub> /mg tissue/hr
Cortex					
216	21	39	17	22	2 8
227	0 5	114	13	101	12 5
303	100	69	23	46	5 8
	3	140	34	106	13 3
	0 5	152	29	123	15 4
316	100	50	11	39	4 9
317	0 5	116	16	100	12 5
318	21	86	21	65	8 1
Medulla					
226	0 5	20	3	17	2 1
304	100	24	6	18	2 3
	3	29	6	23	2 9
	0 5	18	5	13	1 6
Cord					
225	0 5	3	0	3	0 4
228	0 5	4	1	3	0 4
307	100	10	4	6	0 8
	3	10	3	7	0 9
	0 5	8	2	6	0 8

\* 1 mm<sup>3</sup> = 0.004 mg lactic acid

oxygen tension on oxygen uptake. At most points there was considerable overlapping from one tissue to another.

The effect of oxygen tension on glycolysis was quite different from one tissue to another (see Fig 1). In cerebral cortex, as the oxygen tension was lowered there was a progressive increase in glycolysis which was proportional to the decrease in oxygen uptake (Fig 3). The effect was much the same in medulla in the range down to 3 per cent oxygen. From that point instead of

# THE EFFECT OF OXYGEN TENSION ON THE METABOLISM OF CEREBRAL CORTEX, MEDULLA AND SPINAL CORD

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WHEN THE supply of oxygen to the central nervous system falls progressively, normal activity diminishes and irreversible changes occur. For many years differences in response to low oxygen tension have been recognized between cortex, medulla, and spinal cord. The purpose of this study is to investigate possible metabolic bases for these differences.

## METHODS

Twenty-five cats were employed in these experiments. Although full grown animals were not always available, those as small as 1.1 kg did not differ from the adult. Following painless sacrifice, a sagittal cut through the head was made with a guillotine, permitting immediate exposure of the brain. At room temperature (about 25°C) slices were cut from the outer surface of the cerebral cortex, from the medulla oblongata in parasagittal plane, and from the cervical portion of the spinal cord transversely. Oxygen uptake at 38° was measured by the single vessel method of Warburg (16) in vessels of 11 cc capacity containing 2 cc of medium. The medium contained 0.118 M NaCl, 0.0024 M KCl, 0.0017 M CaCl<sub>2</sub>, 0.00066 M MgCl<sub>2</sub>, 0.003 M NaH<sub>2</sub>PO<sub>4</sub>, 0.017 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.011 M dextrose. A high rate of shaking was employed, 160 cycles per min through 1.5 cm. The first reading was taken about 30 min after decapitation, including from 10 to 15 min in the bath for gassing and equilibration, readings were continued for 2 hours. The results are reported on the basis of dry weight of tissue at the end of the experiment. This was of the order of 10, 20 and 35 mg for cortex, medulla and cord respectively. With cord, and to a lesser extent with medulla, the slices sometimes partially disintegrated, the residuum was centrifuged to remove the medium, and weighed separately. This procedure neglects the weight of the precipitated calcium phosphate, about 0.9 mg/cc. The gas mixtures were made from oxygen and nitrogen (the nitrogen contained from 0.3 to 0.5 per cent oxygen) by displacement of saturated CaCl<sub>2</sub> solution from 20 liter carboys, they were analyzed with the Haldane apparatus. Since at 38° water vapor forms 7 per cent of the gas mixture, the oxygen tensions reported in the paper must be corrected by the factor 0.93. Accordingly "100 per cent oxygen" refers to a tension of 0.93 atmosphere. After the removal of the slices the lactic acid in the medium was determined by the colorimetric method of Barker and Summer-son (3). Separate determinations for the preliminary period before the first reading were not performed in each case, but a representative series of values is given in Table I.

## RESULTS

For the most part the rate of oxygen uptake was constant over the two hour period, one consistent variation in rate was the increase from the first to the second hour at the lowest oxygen tensions. Average values for oxygen uptake in oxygen, and lactic acid output at different oxygen tensions are compared in Fig 1. The ratio, cortex: medulla: cord, was 100: 34: 12 for respiration in oxygen and 100: 17: 5 for anaerobic glycolysis.

\* Aided by a grant from the Milton Fund of Harvard University.

have been questioned by Avery, Kerr and Ghantus (2) The peculiar effect of oxygen tension on glycolysis in the medulla is of significant magnitude, and is controlled technically by the results obtained with cortex \*

We have as yet no definite indication of a mechanism that can explain how glycolysis in the medulla could be maximal at an oxygen tension of from

Table 2 Means and standard errors of the means

pO <sub>2</sub>		Oxygen uptake			Lactic Acid output in 2 hr + preliminary period	
		1st hr	2nd hr	Mean	No of cats	mg /g
Vol per cent dry	No of cats	QO <sub>2</sub> = mm <sup>3</sup> /mg /hr		Per cent of value in 100 per cent O		
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Cortex						
100	15	10 5 ± 0 35	10 2 ± 0 27	100	14	52 ± 3 9
21	10	6 7 ± 0 39	7 1 ± 0 32	69 ± 5 3	9	65 ± 6 4
10	8	3 9 ± 0 48	4 5 ± 0 40	42 ± 5 4	7	83 ± 6 2
3	9	2 0 ± 0 11	2 4 ± 0 10	21 ± 1 4	7	121 ± 12 7
0 5	9	0 1 ± 0 12	0 7 ± 0 13	4 ± 0 8	7	138 ± 14 5
Medulla						
100	14	3 5 ± 0 18	3 4 ± 0 18	100	14	18 ± 2 2
21	6	2 3 ± 0 22	2 3 ± 0 19	76 ± 11 6	6	20 ± 3 3
8	7	1 3 ± 0 22	1 6 ± 0 21	50 ± 10 6	7	25 ± 2 5
2	7	0 9 ± 0 14	1 0 ± 0 14	29 ± 5 8	7	32 ± 3 1
0 4	7	0 2 ± 0 09	0 4 ± 0 08	9 ± 2 3	8	23 ± 2 2
Cord						
100	12	1 3 ± 0 10	1 3 ± 0 07	100	12	9 ± 1 0
21	6	1 0 ± 0 16	1 0 ± 0 17	83 ± 10 7	6	8 ± 2 1
8	6	0 6 ± 0 03	0 6 ± 0 04	58 ± 6 8	6	10 ± 1 8
2	6	0 4 ± 0 06	0 4 ± 0 05	37 ± 3 9	6	8 ± 1 4
0 4	5	0 0 ± 0 10	0 2 ± 0 03	10 ± 4 5	6	7 ± 1 1

2 to 3 per cent of an atmosphere instead of zero Nevertheless, the observation is of interest in connection with possible explanations of the delayed post-apneic hypopnea (11), in dogs under light anesthesia deprived of their peripheral chemoreceptors Evidently the motor impulses arising from the respiratory center pass through a maximum after the readmission of air to

\* The following observations should be borne in mind, although they do not contribute directly to an explanation of the effect in medulla Rosenthal and Lasnik (12) noted an increase in anaerobic glycolysis of liver and other tissues following an incubation period in oxygen Ashford and Dixon (1) studied the metabolism of brain cortex in a medium to which M/10 KCl had been added and found an increase in aerobic glycolysis but a decrease in anaerobic glycolysis

rising to a maximum in nitrogen, however, the glycolysis in this tissue fell. In cord, on the other hand, the effect of oxygen tension was slight

### DISCUSSION

The rate of oxygen uptake is the metabolic factor commonly discussed in connection with differences between cerebral cortex, medulla oblongata and spinal cord in sensitivity to anoxia. Recent studies of the metabolism of the brain in the growing mammal (9, 15) have confirmed earlier observations that in the adult, the regions less vulnerable to hypoxia have the lower metabolic rate. Comparison of the rates of respiration and of anaerobic

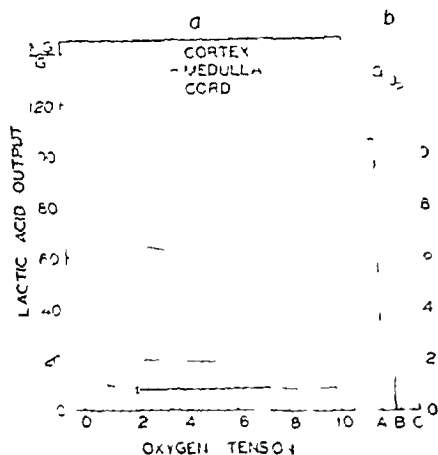


FIG 1 (a) Data from table 2, column 7 vs column 1, oxygen tension in atmospheres  $\times 0.93$  (b) Mean oxygen uptake in oxygen in  $\text{mm}^3/\text{mg/hr}$  where A, B, and C refer to cortex, medulla and cord respectively

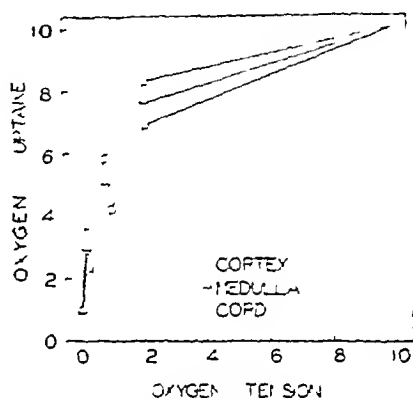


FIG 2 Data from table 2, column 5 vs column 1, oxygen tension in atmospheres  $\times 0.93$

glycolysis in the cat, however, suggests that glycolysis may be a factor of some importance (Table 2). The  $Q_{O_2}$  of cortex in oxygen was three times as great as that of medulla, while the anaerobic glycolysis of cortex was six times that of medulla. This proportionately greater rate of glycolysis of cortex might affect the sensitivity of cortex to anoxia through depletion of glucose or accumulation of acid. Even as the medulla oblongata has an advantage in this regard over cerebral cortex, so the spinal cord appears to have an advantage over medulla when one compares aerobic and anaerobic glycolysis. Oxygen tension had relatively little effect on glycolysis in cord, whereas in medulla glycolysis was 28 per cent higher in nitrogen than in oxygen. The relatively high aerobic glycolysis in cerebral cortex has already been discussed (5). Glycolysis in the medulla has been subjected to little if any direct study since that of Haldé, Ward and Woo (8), and their results



the behavior of slices and suspensions to inadequate diffusion of oxygen into the slice. If diffusion into the slice accounts for the high critical oxygen tension shown in Fig 2, then the data for 100 per cent oxygen are probably most nearly comparable to the situation *in vivo* when air is being breathed, for glycolysis is at a minimum in oxygen *in vitro* and in air *in vivo*. If, on the other hand, Fig 2 is interpreted to mean that *in vivo* brain metabolism could increase, if a greater partial pressure of oxygen were supplied in the inspired air, the results are significant in a consideration of the toxic action of high concentrations of oxygen. Using a medium free of Ca and Mg ions Elliott and Libet (7) described a decline in rate with time that was more marked in oxygen than in air. In our medium a significant decline in the rate from the first to the second hour (Table 2) was not observed.

The intimate connection between respiration and glycolysis in cortex is illustrated in Fig 3, for when oxygen tension varies, the relationship between the two processes is linear. The implication of this is to limit the generality of the results obtained with retina in bicarbonate medium (10, 13), in which the Pasteur enzyme appeared to influence glycolysis but not respiration. The relationship between respiration and glycolysis observed in cerebral cortex is also found in bone marrow (17), and in retina in phosphate medium (6).

#### SUMMARY

The rates of oxygen uptake of cortex, medulla and spinal cord were in the ratio 100 : 34 : 12, whereas the ratio for anaerobic lactic acid production was 100 : 17 : 5.

The oxygen uptake of all three tissues was sensitive to oxygen tension. The shape of the oxygen uptake-oxygen tension curve was essentially the same for the three tissues.

The anaerobic lactic acid production was twice as great in cortex as in medulla per unit of oxygen uptake in oxygen. Lactic acid production was sensitive to oxygen tension in cortex and medulla but not in cord. These differences in lactic acid production are offered in partial explanation for the variations in resistance to anoxia from one region to another within the central nervous system.

In cortex, lactic acid production and oxygen uptake varied inversely when the oxygen tension was altered.

In medulla, surprisingly enough, lactic acid production was maximal at 2 to 3 volumes per cent oxygen. The possible relationship of this to a previously described respiratory phenomenon was mentioned.

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the dogs Glycolysis in the medulla follows a parallel course This possible relationship needs to be investigated further, since it may well provide a better understanding of the problems involved in chemical stimulation of respiration

The oxygen tension-oxygen uptake curves are described by the equation of Tang (14),  $P/A = K_1 + K_2P$ , where  $P$  is oxygen tension,  $A$  the rate of respiration,  $K_1$  the intercept and  $K_2$  the slope When  $P/A$  was plotted against  $P$  (Fig 4), linear relationships were obtained Tang cited 17 vari-

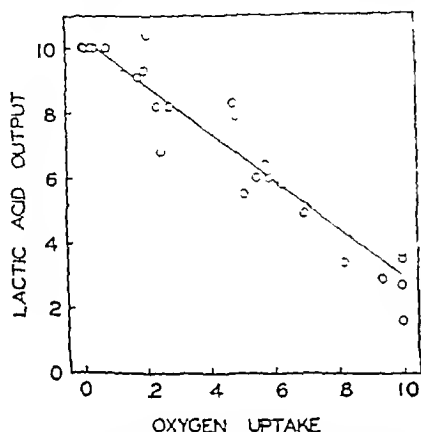


FIG 3 Cerebral cortex Data from 7 cats, tissue from each animal was studied at different oxygen tensions Each point represents the oxygen uptake at an intermediate oxygen tension as a fraction of the oxygen uptake in oxygen for that animal, and the lactic acid output at the same oxygen tension as a fraction of the lactic acid output in nitrogen

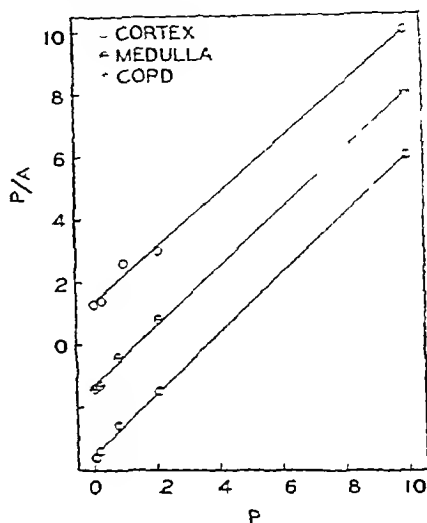


FIG 4 Data from table 2,  $A$  from column 5,  $P$  from column 1, oxygen tension in atmospheres  $\times 0.93$  The intercepts on the  $P/A$  axis were 0.13, 0.07 and 0.05 for cortex, medulla and cord respectively

eties of biological material the data for which were described by this equation The curves for brain *in vitro* (Fig 2) represent a possible deviation from the behavior of brain *in vivo* In cerebral cortex, oxygen uptake was depressed significantly in 21 per cent oxygen as compared with 100 per cent, whereas *in vivo*, changes in metabolic and mental activity are not observed until the oxygen tension in the inspired air is lower (4) The discrepancy between the  $Q_{O_2}$  of cortex *in vivo* and *in vitro* has been discussed elsewhere (5)

Elliott and Libet (7) in studying the oxygen uptake of rat brain in air and in oxygen, found that the substitution of oxygen for air had no effect on the initial rate of a homogenized suspension of whole brain, whereas with slices of cortex there was a 42 per cent increase This agrees with our figure of 45 per cent for cortex (Table 2)\* They attributed the difference between

\* This appears to be true also of medulla and cord but since the differences in 100 and 21 per cent oxygen are smaller than in cortex, a larger body of data would be required to establish the point for them



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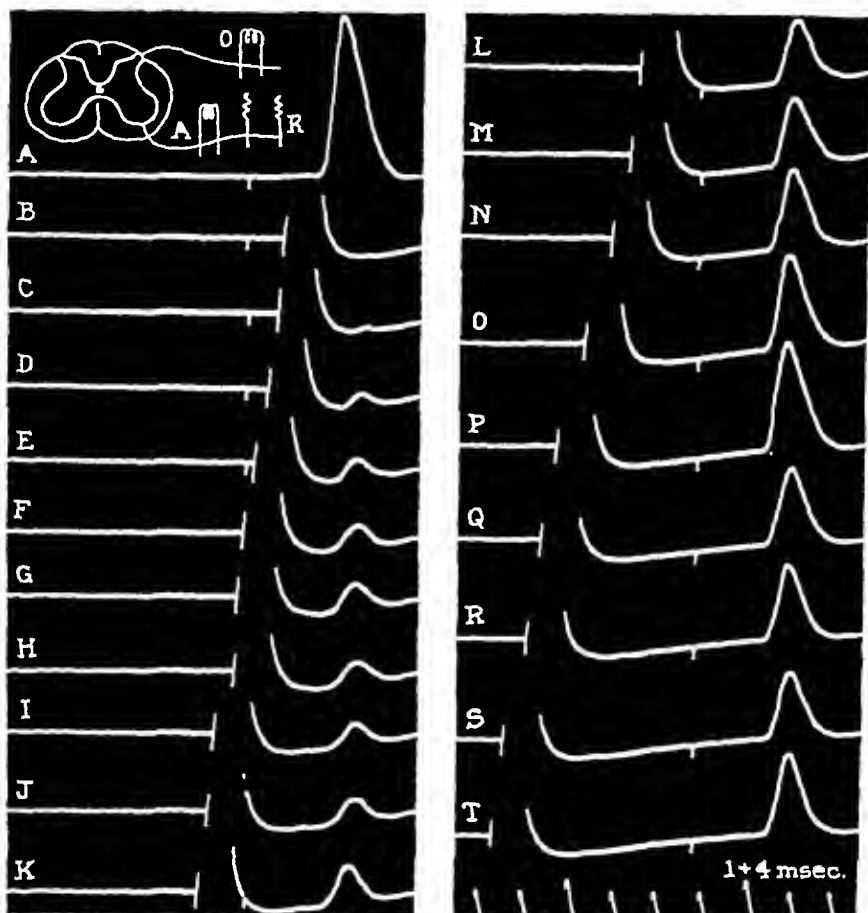


FIG 1 Segmental (S1) reflex preparation. The recovery of reflex response following an antidromic volley. The inset serves to illustrate the disposition of stimulating and recording electrodes. The orthodromic, or reflex, volley is evoked by stimulation through electrodes A. The antidromic volley is evoked by stimulation through electrodes A on the ventral root. Records are obtained by means of electrodes R on the ventral root distal to the A electrodes. Record A: reflex response in isolation. Records B-T: reflex response with combined orthodromic and antidromic stimulation. The antidromic shock falls progressively earlier with respect to the orthodromic shock which is fixed in position on the sweep. Note two stage recovery with "plateau" period (records F-J) during which the reflex remains relatively constant in size. Time in 1 and 4 msec intervals.

times differ slightly due to the different position of the stimulating and recording leads on the ventral root.

Ventral root conduction time for the antidromic volley is obtained by stimulating with the A electrodes (Fig 1, inset) and recording the motoneuron response by means of a microelectrode, the interval between the A shock and the onset of negativity at the microelectrode denoting the value for the correction. Ventral root conduction time for the orthodromic volley

# THE INTERACTION OF ANTIDROMIC AND ORTHODROMIC VOLLEYS IN A SEGMENTAL SPINAL MOTOR NUCLEUS

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A MAXIMAL antidromic volley (1) backfired into a segmental spinal pool of motoneurons fails to block a reflex volley completely unless the opposed volleys clash in the motor axons. The following experiments describe, and attempt to account for, this finding.

In Fig. 1 are presented the results of an experiment in which an antidromic volley and an orthodromic (reflex) volley pertaining to two-neuron-arc reflex systems are caused to interact. The reflex of the first sacral segment is utilized in this experiment, as in most of the others, for the ventral root of this segment in the cat is of satisfactory length for the placing of the required electrodes. Record 1A illustrates the reflex volley recorded in isolation from the ventral root. In records 1B-1T, antidromic and reflex volleys are combined, the orthodromic shock initially preceding and subsequently trailing the antidromic shock. The orthodromic shock remains fixed on the time axis of the successive records.

When the orthodromic shock precedes the antidromic shock by 0.75 msec (0-0.75-A) as in Fig. 1B, the orthodromic response is completely blocked, as it is with the use of greater stimulus intervals until the orthodromic response begins to reach the recording leads before the initiation of the antidromic volley. A response to the orthodromic shock is first secured in Fig. 1C, recorded with a shock separation of (0-0.6-A). The orthodromic response increases until (A-0.1-0) in record 1F, following which it remains constant until (A-0.9-0) in record 1J, after which a second rise in amplitude is realized.

In earlier experiments employing antidromic conditioning (1, 5, 8) the first orthodromic responses were obtained with shock separations of (A-0.43-0) to (A-2.3-0), which would correspond approximately to records J and O in the series of Fig. 1. The initial step in recovery, therefore, has not been described previously.

Figure 2, curve A, illustrates graphically another experiment similar to that presented in Fig. 1. On theoretical grounds the ideal zero for a recovery curve employing synaptic excitation would be that at which the antidromic volley coursing over the somata of the motoneurons (6) reaches the points at which the postsynaptic reflex responses are evoked at the exact time that the orthodromic volley reaches those points. One can appreciate that the ideal zero is not readily attainable in practice. However, a useful correction can be made by allowing for conduction in the motoneuron axons, which can be measured satisfactorily. Separate measurements are required for the ventral root conduction time of the reflex and antidromic volleys, as these

is obtained by inserting stimulating electrodes into the ventral horn in such a way that the motoneurons may be stimulated directly by an electrical shock, the response being recorded by means of the R electrodes. When the orthodromic shock antecedes the antidromic shock by an interval equivalent to the total reflex latency minus the sum of the ventral root corrections, the opposed volleys should clash at the junction of axon and soma of the motoneurons. Zero on the scale of ordinates is set so as to represent this interval of shocks. In consequence the time scale represents, within the limits of measurement, the interval between the arrival of the antidromic volley and the arrival of the orthodromic volley at the axon-soma junctions. The arrow indicates the status at the coincidence of the two shocks, orthodromic and antidromic.

When the antidromic and orthodromic volleys coincide at the axon-soma junction, as indicated by the origin of curve 2A, the orthodromic volley is obliterated. The orthodromic volley, however, need reach the junction only 0.1 msec later than the antidromic volley to be successful in eluding to some extent total refractoriness. The orthodromic volley, by the time it reaches the axons of the motoneurons, has been subjected to considerable conduction and to synaptic transmission, with the certain result that it is more dispersed than the antidromic volley arriving at the same point. In consequence it may be that only the last impulses of the dispersed orthodromic volley to arrive at the axon elude refractoriness to reach the recording leads when such short intervals are employed. Nevertheless it is apparent that the orthodromic volley passing through the reflex arc is capable of exciting the motoneuron axon for a period approximately equal in duration to the absolutely refractory period of the axon. The added delay in the reflex response, which is easily seen in Fig. 1, presumably is due to refractoriness and takes place at the axon junction. As the reflex volley falls later the response increases sharply to a plateau which is maintained for approximately 1 msec before a further increase in the response takes place.

The observations of Fig. 1 and 2A indicate that some impulses in the orthodromic volley are not blocked by the antidromic volley when the two volleys meet in the motoneurons at some point central to the axon junction, even though the antidromic volley blocks the orthodromic volley completely when they meet in the axons of the motoneurons. In attempting to account for these observations it is germane to consider some aspects of the response of the motoneuron somata to antidromic activation.

The action potential of the motoneurons, excited by antidromic shocks and recorded by the use of a microelectrode appropriately situated, consists of an initial positive deflection signalling the approach of the volley in the axons, followed by a large negative deflection, this in turn being followed by a more prolonged positive deflection (6). The motoneuron potential as recorded in the sacral spinal cord may be seen in Fig. 2C. The large negative "spike" potential of the motoneuron somata, in contrast to the spike potential of the axons, is labile (7), it is easily blocked by asphyxia (7), it is pro-

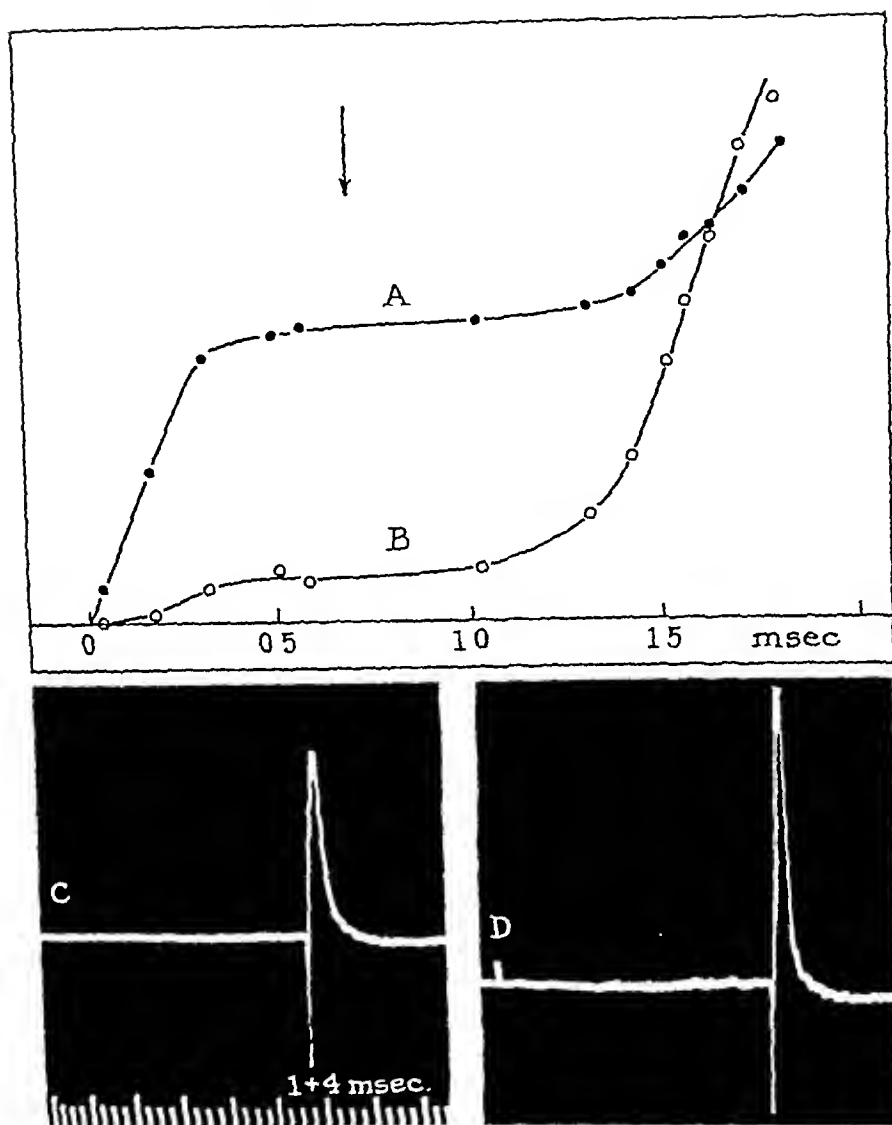


FIG 2 A recovery curve constructed from observations such as those presented in Fig 1 Ordinate—amplitude of the reflex response in arbitrary units Abscissa—time The arrow indicates coincidence of the antidromic and orthodromic shocks To the left of the arrow the orthodromic shock leads, to the right of the arrow the antidromic shock leads Time is plotted according to the conduction corrections explained in the text B recovery curve constructed in the same manner as curve A All details for curves A and B are identical with the single exception that the antidromic volley employed for the observations plotted in curve B was preceded by a single shock to the ipsilateral brachial plexus Note reduction of the initial recovery stage C potential recorded by microelectrode from the motoneurons of the S1 segment as the consequence of a single maximal antidromic volley D as in C except that the antidromic shock is preceded by a single shock to the ipsilateral brachial plexus with the result that the soma response (7, 8) of the motoneurons is facilitated Time for C and D below record C in 1 and 4 msec intervals



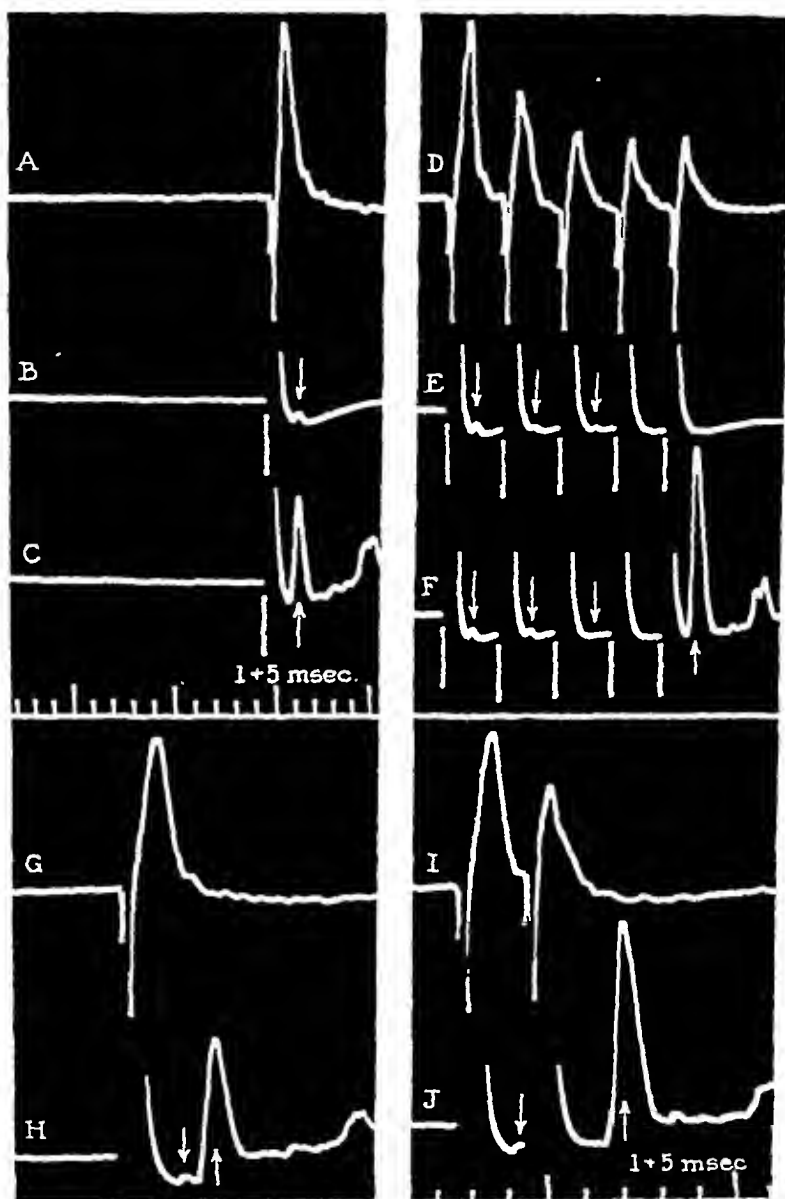


FIG 3 Changes in the soma response of the motoneurons, in the recurrent motoneuron discharge and in the initial stage reflex discharge resulting from repetitive antidromic stimulation. Recurrent discharges are identified by means of arrows directed downward, initial stage reflex discharges by means of arrows directed upward. Further description in text. Time for records A-F in 1 and 5 msec intervals is below record C. Time for records G-J in similar units is below record J.

gressively reduced on tetanic stimulation at frequencies which the axons follow with ease, it may be facilitated and inhibited (10)

The fact that the soma response to a maximal antidromic volley may be facilitated indicates that the antidromic volley fails to enter the somata of some of the motoneurons or that, on entering, it produces effects graded in extent or intensity. Of these possibilities, the first, failure of the antidromic volley to extend beyond the axons into the somata of some of the motoneurons available to the orthodromic reflex volley, would account for the initial rise and plateau of the recovery experiments illustrated in Fig 1 and 2, for in these motoneurons the orthodromic volley would not encounter refractoriness until reaching the axon junction. An explanation of the initial stage in recovery based on the preceding considerations may be put to test by the simple expedient of facilitating or depressing the response of the motoneuron somata to the conditioning antidromic volley

In order to compare the recovery curve of reflex responses in association with resting and facilitated antidromic volleys it is useful to procure a facilitating background activity which is relatively constant over a period of several milliseconds. A single shock to the brachial plexus (3) provides an admirable source of facilitatory impulses for it satisfies the present requirements. Fig 2C illustrates the motoneuron potential recorded by means of a microelectrode in the resting spinal cord, the stimulus is a single maximal antidromic shock. Fig 2D shows how the soma potential is greatly augmented and slightly synchronized by the activity resulting from the brachial plexus stimulation. The initial positive deflection, signalling the approach of the antidromic volley in the axons, is unaltered from record C to record D.

Figure 2, curve A, as noted before, illustrates graphically the initial stage in recovery of reflex response. Curve B is constructed in a similar manner from observations obtained under identical conditions with the single exception that the antidromic volley is preceded, as in 2D, by a single shock to the brachial plexus. A comparison of curves A and B reveals that the size of the reflex responses is severely reduced for the duration of the initial stage or plateau. Subsequently the action of the brachial plexus stimulation is to facilitate the reflex volley as it does in the absence of the antidromic volley (3), the reflex volley, of course, still being of subnormal size. Thus the greater the soma response to the antidromic volley the less spectacular are the reflex responses of the initial stage in recovery.

If the reduction in size of the initial stage reflex responses illustrated in Fig 2 is indeed due to increase in the response of the motoneuron somata to the antidromic volley, then it follows that the initial stage reflex discharges should increase in size as the response of the motoneuron somata to the antidromic volley is depressed. Depression of the soma response of the motoneurons may be attained by repetitive antidromic stimulation (10). Before discussing the effects of repetitive antidromic stimulation it is appropriate to consider another type of motoneuron discharge to the periphery which occurs during the period of central activity following an antidromic shock

*On the question of refractoriness of the motoneuron somata* The initial stage reflex discharge appears first at the end of absolute refractoriness in the motoneuron axons, or shortly thereafter, and while some, but not all, of the motoneuron somata are still occupied by the antidromic volley. The initial stage reflex discharges are obtained by transmission through those motoneurons in which the antidromic volley presumably is blocked at the axon junction. The early initial maximum and plateau show that within a few tenths of a msec virtually all of the motoneurons available to the orthodromic volley by reason of the failure of the antidromic volley are recruited to the service of the orthodromic volley. Approximately 1 msec after the end of absolute refractoriness at the axon, the plateau of the initial stage reflex response ends and a second stage of recovery begins. The second stage of recovery appears to represent the beginning of transmission of the reflex volley through those motoneurons in which the antidromic volley enters the soma. One may conclude that conduction in the motoneuron soma is accompanied by refractoriness, and that on the occasion of antidromic activation the motoneuron is not open to synaptic activation for approximately 1 msec after the axon has recovered from absolute refractoriness. This statement must not be taken to mean that the refractory period of the soma is longer by 1 msec than that of the axon, since the upper limit for a refractory period of motoneuron somata has been set at 0.6 msec (4) which is virtually that of the axons.

*Other considerations* If the antidromic impulse dies at the axon-soma junction of some motoneurons (cf 7), which appears to be the case, then one might expect the same changes to take place in the somata of those motoneurons as are known to take place beyond a block in nerve (2, 6). On this supposition the somata of the motoneurons in which the antidromic volley dies would be more excitable than in the "resting" state and would accordingly be more accessible to the reflex volley than they would be in the absence of the antidromic volley. On the other hand the activity in the fully occupied motoneurons might affect adversely the transmission of impulses in the orthodromic direction through the motoneurons that are not fully occupied by the antidromic volley (9). Considerations such as these give some hint of the complexity of the central disturbance created by an antidromic volley. In general it seems that antidromic volleys should be employed with due caution, and that oversimplified assumptions as to the central effect of an antidromic volley should be avoided. There can be little question but that an antidromic volley does more than interpose a refractory period in the path of a reflex.

#### SUMMARY

A maximal antidromic volley backfired into a segmental spinal pool of motoneurons fails to block a reflex volley completely unless the opposed volleys clash in the motor axons. This is apparently due to the failure of the antidromic volley to conduct from the axon into the soma in some of the

Frequently, among spinal motoneurons at any rate, the transmission of an antidromic volley is followed by the discharge of a small centrifugal volley in the absence of any other specific stimulation (9) The central latency for this "pseudo-reflex" volley, or recurrent discharge as it will be called, is somewhat less than 1 msec Furthermore the recurrent discharge occurs only in motoneurons that are occupied by the antidromic volley, it may be inhibited by appropriate orthodromic activity In appearance the recurrent volley is not unlike the initial stage reflex volley, but the two may be differentiated quite easily by experiment

The observations of Fig 3 illustrate the behaviour of the initial stage reflex discharge when an orthodromic volley is associated with single and repetitive antidromic volleys Shown also is the differentiation between recurrent motoneuron discharges and initial stage reflex responses Record A of Fig 3 shows the motoneuron potential recorded by a microelectrode and resulting from a single antidromic volley In 3B is recorded, from the ventral root, the antidromic volley followed closely by the recurrent motoneuron discharge In Fig 3C, a single dorsal root shock is delivered synchronously with the antidromic shock. The initial stage reflex discharge may be seen in 3C occupying the same relative position as the recurrent discharge in 3B Fig 3, A, B, and C form control records for the consideration of 3D, E, and F in which five antidromic volleys are utilized

Figure 3D records the motoneuron potentials evoked by five rapidly repeated antidromic volleys It will be seen that the soma potential is progressively reduced (cf 10) On the contrary the initial positive deflection, signalling each volley approaching in the axons toward the microelectrode, retains its maximal size throughout In 3E is shown the result of five antidromic volleys as recorded on the ventral root By comparing 3E with 3D it will be seen that the recurrent motoneuron discharges decrease in parallel with the soma potentials and reach final extinction Examination of 3F, however, shows that the initial stage reflex discharge is differently affected In 3F, as in 3C, a dorsal root shock is delivered simultaneously with an antidromic shock, in this case with the final antidromic shock of the series Again as in 3E, the progressive decrease in the recurrent discharges is evidenced, but the initial stage reflex discharge is greatly increased over its size following a single antidromic volley

Figure 3 (G-J) repeats the experiment just described with the exception that the orthodromic shock is delivered approximately 0.6 msec later than the corresponding antidromic shock By this device the reflex discharge is kept within the initial stage or plateau period, but it is sufficiently late to clear entirely the recurrent motoneuron discharge, thus avoiding any confusion between the two discharges Records G and I of Fig 3 illustrate the soma potentials recorded following a single and two antidromic volleys respectively Comparison of 3H and 3J shows that the recurrent discharge drops out, whereas the initial stage reflex is enhanced as the soma potential is depressed after an antecedent antidromic volley



motoneurons In consequence the recovery curves obtained by the use of synaptic stimulation reveal two stages, the first rise being referable to axonal recovery of their motoneurons the somata of which are not activated by the antidromic volley, the second to recovery of synaptic transmission through the motoneurons in which the antidromic volley does occupy the somata

The prominence of the initial stage depends upon the number of motoneurons in which the antidromic volley fails to evoke a soma response

Conduction in the neuron soma is accompanied by refractoriness

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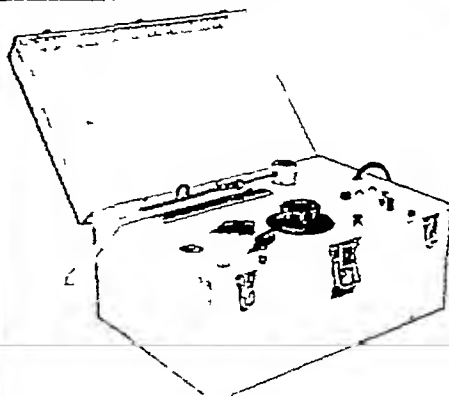
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at a point farther from the cut end of the root lowered both ipsilateral and crossed thresholds to a common level comparable to that obtained from the root of the opposite side

In terms of crossed inhibition the evidence of asymmetry is scarcely less

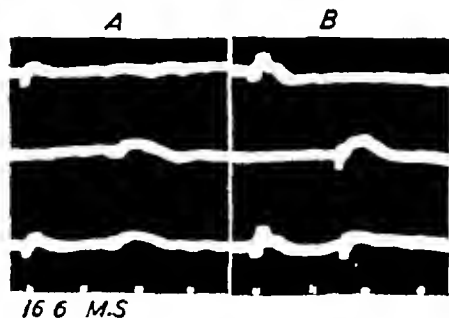


FIG 1 A Lead from the right dorsal surface of the cord Conditioning shock of 110 arbitrary units of stimulus strength to a left dorsal root Test shock of 30 units to a right dorsal root, both shocks at interval of 27 msec Note the absence of inhibition even when a weak test volley is employed On this side there was no visible inhibition of digital flexion

B Lead left Conditioning shock right 110 units test shock left 60 units, both shocks at interval of 28 msec Note the marked inhibition of the cord potential The movement of digital flexion was inhibited completely with this combination of stimuli *Macaca mulatta* 3 kg right

hemisection Dec 4, 1940, transection and recording Dec 26, 1940

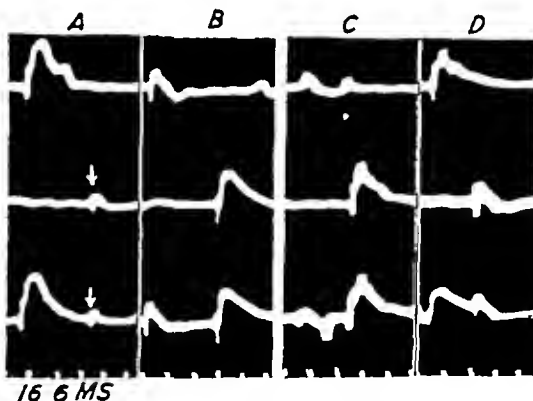
meager This was tested on both sides in nine experiments, in only one of which was there a significant difference, the previously paretic side proving the more potent source of inhibition of both internuncial potential and reflex (Fig 1) The more usual symmetrical situation (in this instance with shock to the crossed potential and no interaction) is illustrated in Fig 2

FIG 2 A Right dorsal lead, conditioning shock of 58 units to a right dorsal root, test shock of 58 units to a left dorsal root, both shocks at interval of 37 msec

B The same combination of stimuli led from the left Shock interval 46 msec

C Right lead, conditioning shock of 34 units to a left dorsal root, test shock of 18 units to a right dorsal root, both shocks at interval of 32 msec

D The same combination of stimuli led from the left, shock interval 29 msec Note the evidence of spinal shock in the crossed potentials and the absence of inhibition in all four columns *Macaca mulatta* 3 kg right hemisection May 22, 1941, transected and recorded June 10, 1941



Far more predictable is the order of recovery of crossed reflexes The severity of spinal shock in this field proved a serious limitation, since it was essential to record soon after transection in order to obtain the highest available degree of asymmetry Consequently, only two of our monkeys so recorded developed a crossed response In both instances it was elicited in the musculature of the previously paretic limb by a stimulus on the previously intact side Of special interest is an animal recorded 41 days after hemisection but only a few hours after transection This is the only instance we

# THE MONKEY (*MACACA MULATTA*) AFTER HEMI-SECTION AND SUBSEQUENT TRANSECTION OF SPINAL CORD\*

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(Received for publication January 23, 1943)

## INTRODUCTION

THE REFLEX picture resulting from hemisection followed at a suitable interval by transection has been described by Fulton and McCouch (1) in the case of a single baboon and the cord potentials in the monkey have been discussed by the present authors (5) Since certain of the results we reported in 1940 have not been confirmed uniformly in a larger series, we are impelled to reopen the subject

## RESULTS

In accord with earlier results (1, 5), all of our fifteen animals recovered reflex activity more rapidly upon the side of previous hemisection On the

Table 1 Ratio of Thresholds  $\frac{\text{Cord Potential}}{\text{Reflex}}$

Date	Chronic	Acute	
3/ 7/39	1 0-0 89	0 42	Both legs dissected
6/20/40	0 47	0	" " "
12/26/40	0 95	0 51-0 36	Legs not dissected
3/ 4/41	0 74	0 37	" " "

other hand, internuncial potentials recorded from dorsal or dorsolateral surface leads yielded responses that rarely showed significant differences between the two sides When asymmetry did occur, it was far less marked in the potentials of interneurons than in the corresponding reflex actions These points are indicated by the difference in threshold between cord potential and ipsilateral flexor reflex presented in Table 1 in the form of a ratio In the previously monoplegic extremity this may approximate unity On the previously intact side, the value for the threshold of the internuncial potential divided by that for the ipsilateral flexor reflex lies between 0.51 and 0

A high threshold for the crossed cord potential may occur on either side or upon neither and is frequently associated with a lesser rise in threshold on the ipsilateral side In one instance, replacement of stimulating electrodes

\* Aided by a grant from the National Committee for Mental Hygiene of the Supreme Council Thirty Third Degree Scottish Rite Masons, for research in dementia praecox and by the John and Mary R Markle Foundation

Sherrington's fundamental experiment of successive transections, of which the second failed to induce a degree of depression comparable to that ensuing after the first (6) In this regard we merely confirm for the monkey the result of Fulton and McCouch (1) in a single baboon Reflexes are less depressed by transection on the side of the previous hemisection because the majority of the descending axons which facilitate their arcs had been severed by the preceding hemisection and thus a longer interval for recovery was obtained

The crossed effects are less obvious Here the result hinges upon the relative depression of the various neurons in a complex reflex path In previous papers (2, 7) evidence has been advanced for the proposition that in the monkey spinal shock depresses the cells of the ventral horn (presumably the motoneurons) far more deeply than the interneurons situated more dorsally This conclusion is supported by the present findings If the last cells to recover be the motoneurons, crossed reflexes should find their earliest motor expression upon the side of previous hemisection where recovery is more advanced and in those units which show least depression Such results as we have confirm this expectation Thus digital flexion, which is the first ipsilateral reflex to return is likewise the first crossed response

In the case of crossed excitatory reflexes just considered, it is essential for the interneurons on the side suffering the greater depression to recover sufficiently to discharge the contralateral motoneurons With crossed inhibitory reflexes such internuncial shock may be a significant factor in the order of recovery Another may lie in the high susceptibility to inhibition of the motoneuron when its excitability is low (3) Both influences favor inhibition from a source on the chronic side of previous hemisection acting upon the motor cells of the more depressed side which has suffered almost its entire quota of shock after transection Here again our single significant instance of such asymmetry accords with expectation

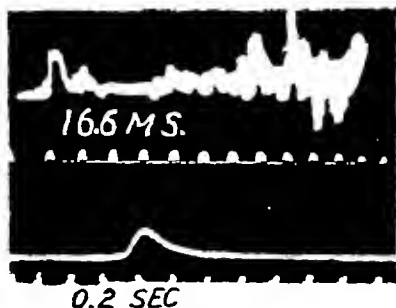
Consonant with the evidence of the relatively light degree of shock to interneurons is the frequent absence of asymmetry in their potential, even in its crossed component A high threshold for the crossed potential, though exceptional, may occur on either side and in one instance was lowered by adjustment of stimulating electrodes We would not imply, however, that it never occurs as a result of asymmetry in depression of interneurons That such was actually the case in the instance cited in a previous paper (5) is strongly suggested by its long latency in that animal In a situation in which the result is determined in part by the magnitude of the volley in crossing neurons, in part by the depression of the cells on which they impinge, either of these opposing factors may prove dominant and hence it is scarcely surprising that the threshold should prove unpredictable

#### SUMMARY

A series of 14 monkeys (*Macaca mulatta*) and one *Macacus mordax* have been studied after hemisection and subsequent transection of the spinal cord

have observed of the presence of a crossed reflex giving gross movement in the absence of any visible ipsilateral response. There had been no dissection of the legs. Save for a laminectomy and section of a dorsal root on each side, the animal was intact below the level of transection. Various strengths of stimulus were employed of which a wide range yielded flexion of the contralateral toes. Yet at no time was there movement or visible muscular con-

FIG 3 Crossed extension of leg of chronic side. Upper record: Cord potential from left dorsal lead. Lower record: myogram of right quadriceps femoris. The records are not temporally aligned. The stimulus induced a single volley in the left sciatic nerve including delta fibers. Presumably the abrupt onset of the potential is associated with the ipsilateral flexor reflex, the later, progressively increasing response with the crossed extension recorded in the myogram. Time in cord potential in units of 16.6 msec. Time in myogram in units of 0.2 sec. Tension developed in reflex 130 g. *Macacus mordax* 3.3 kg. right hemisection Oct. 18, 1939, transection Nov. 9, 1939, recording Nov. 14, 1939.



traction upon the side of stimulation. Only by electrical records from muscle was a faint ipsilateral reflex demonstrable. In sharp contrast was the response from the root of the previously paretic side: sharp contraction of ipsilateral semitendinosus and no visible crossed response.

The other case of crossed contraction occurred five days after transection, when extensor motoneurons of the previously hemiplegic leg had recovered sufficiently to give the crossed response of quadriceps femoris shown in Fig. 3.

In these two animals the relation of the type of the crossed reflex to the interval after transection raised the question whether in monkey as in cat (4) crossed flexion may recover from spinal shock earlier than crossed extension. This suspicion was confirmed in a third monkey in which the crossed reflexes were elicited by stimulating electrodes in the pads of the feet almost daily throughout the period of asymmetry. Crossed flexion of toes occurred 5 hours after transection and persisted for 7 days. It responded to single induction shocks as well as to repetitive stimulation. Crossed extension of proximal joints with flexion of digits was first obtained two days later. During the following week the crossed extensor response increased and crossed flexion of digits decreased progressively. Nine days after transection crossed extension occurred without movement of digits, although digital flexion was still readily induced as an ipsilateral reflex. As in the two previous cases, the crossed reflexes were more readily elicited in the chronic extremity from a stimulus on the acute side than vice versa.

#### DISCUSSION

The monkey in which hemisection is followed after a suitable interval (1) by transection offers a useful preparation for the localization of spinal shock. So far as ipsilateral reflexes are concerned, the result was predictable from



Reflex recovery was always more rapid in the previously paretic extremity

Three animals developed crossed reflexes on the chronic side in response to stimulation on the acute side. Crossed flexion of digits was recorded a few hours after transection, crossed extension of the leg two or more days later.

Crossed inhibition may be more effectual when driven from the chronic side affecting the acute side than vice versa.

Only exceptionally is asymmetry reflected in internuncial potentials, never to the degree obtaining in the corresponding reflex responses.

The significance of the asymmetry observed is discussed in relation to spinal shock.

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This procedure led with great regularity to a loss of 80 to 90 per cent of the c r in about 3 to 4 days. After this level had been established for at least 6 days, the rats were subjected to insulin hypoglycemia in order to attempt a restoration of the c r.

Four to 10 units of insulin (Lilly)\* were injected intraperitoneally in two divided doses at an interval of one hour. After the hypoglycemic effects described later had taken place, normal blood sugar and behavior were reestablished by injection of 5 cc of 8 to 12 per cent glucose.

## RESULTS

Since insulin hypoglycemia tends to restore inhibited c r it was first necessary to determine whether or not inhibited c r recover spontaneously. Numerous experiments performed by Kessler and Gellhorn as well as by the present authors show that this is not the case. The following series of experiments illustrates this finding.

Three rats in which the c r had been established and maintained at 90 per cent for 3 days were tested for a period of 25 days daily by 10 non-reinforced c s which were preceded by two reinforced c s. This procedure led to an inhibition of the c r in 3 to 5 days. Hereafter the c r remained at a level of 0 to 10 per cent for 25 days. Only on one day was it found in one animal that the c r rose to 20 per cent. On the basis of these and similar experiments it is concluded that a recovery of more than 20 per cent is significant.

The main group of experiments comprises 22 rats in which the c r to the bell had been inhibited by lack of reinforcement. The administration of insulin resulted in a restoration of the inhibited c r, to varying degrees, lasting several days to several months. In 5 animals these effects could not be obtained but in most instances these animals died before the insulin test could be repeated several times. It is worthy of note that in the last 11 animals no failure was observed.

Two factors appear to be of importance for the recovery of inhibited c r by insulin hypoglycemia: (1), the degree of cerebral depression seems to play a role, (2), the results seem to depend to a certain extent on the method of inhibiting and testing the animal. It was mentioned in the preceding section that inhibiting and testing was done by having a period of 10 non-reinforced c s preceded by one or two reinforced stimuli (bell+shock). It is our impression that the use of two reinforced stimuli is of definite advantage. This procedure does not prevent gradual abolishment of formerly established c r and still maintains the animal during the period of complete inhibition of the c r in a sufficient degree of alertness to permit recovery. It is with this method that a 100 per cent success was achieved in the last 11 animals although in control animals not subjected to insulin the c r never recovered spontaneously.

The description of a few characteristic experiments will show that the recovery depends also on the severity of the hypoglycemic syndrome. Hypoglycemic symptoms occurring within two hours following the injection of insulin may be classified into three convenient groups: (1) insulin depression characterized by absence of spontaneous movements, diminished tone of

\* Kindly supplied by Eli Lilly and Company

# THE EFFECT OF INSULIN HYPOGLYCEMIA ON CONDITIONED REFLEXES

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(Received for publication February 5, 1943)

KESSLER and Gellhorn (4) showed recently that convulsions induced by metrazol or by electroshock led to the restoration of previously inhibited conditioned responses. After several injections of metrazol or after electroshock conditioned responses which had been completely inhibited by lack of reinforcement were restored up to about 70 per cent. The period of restoration lasted for several days during which conditioned responses gradually declined. The effect could be shown repeatedly in the same animal.

An investigation of the effect of hypoglycemia under similar conditions seems to be of great interest for 3 reasons: (i) to increase our knowledge of the effects of insulin hypoglycemia on the brain in chronic experiments, (ii) in order to determine whether the restoration of inhibited conditioned responses results from convulsions only, (iii) in order to obtain, if possible, further material suggesting the fundamental similarity of the physiological mechanisms involved in various forms of shock therapy (1, 2, 3).

The experiments reported in this paper show indeed that insulin hypoglycemia may restore, to a surprising degree, previously inhibited conditioned responses.

## METHOD

The experiments were performed on more than 40 rats, mostly male, of 200 to 250 g weight. The same apparatus as described by Kessler and Gellhorn (3) was used. It consisted of two compartments separated by a low partition. The floor of the compartment was made of copper wire grid which could be charged through a General Electric Variac. A few trials were sufficient to cause the rat to jump from one compartment into the other in response to the electric shock (about 40 V) applied to the grid. The response consisted of two integrated movements: first, the rat jumped across the partition, then turned around completely and again faced the partition in readiness for another jump.

After the unconditioned response (u.r.) had been established in a few trials the conditioning process was carried out as follows: a bell was sounded for two seconds and was followed by an electrical shock. The sound of the bell was continued to the end of the electrical stimulation. To avoid fatigue, not more than 25 to 30 reinforced conditioning stimuli (bell plus shock) were applied in one session. The experiments were performed daily and in general 70 to 120 stimuli (bell plus shock) were adequate to establish a conditioned response of 90 to 100 per cent. This response was then maintained for three successive days, to insure a thorough retention of the conditioned response (c.r.). This was accomplished by subjecting the animals to 7 to 12 conditioned stimuli (c.s., bell) reinforced by unconditioned stimuli (u.s., shock) before applying the test series of 10 non-reinforced c.s.

Hereafter the c.r. was inhibited by the daily application of 10 unreinforced c.s. (inhibition by lack of reinforcement in the sense of Pavlov). However, this test was always preceded by one or, in the majority of the experiments, two reinforced stimuli. In the latter case one was applied while the animal was in compartment A and the other in the compartment B in order to avoid the formation of positional habits.

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\* Aided by a grant from the John and Mary R. Markle Foundation.



inhibited responses up to 100 per cent This experiment suggests that insulin coma is more effective in restoring the c r than is the hypoglycemic "depression" Moreover, it appears probable that insulin coma exerts a cumulative effect on the central nervous system since the coma becomes more effective

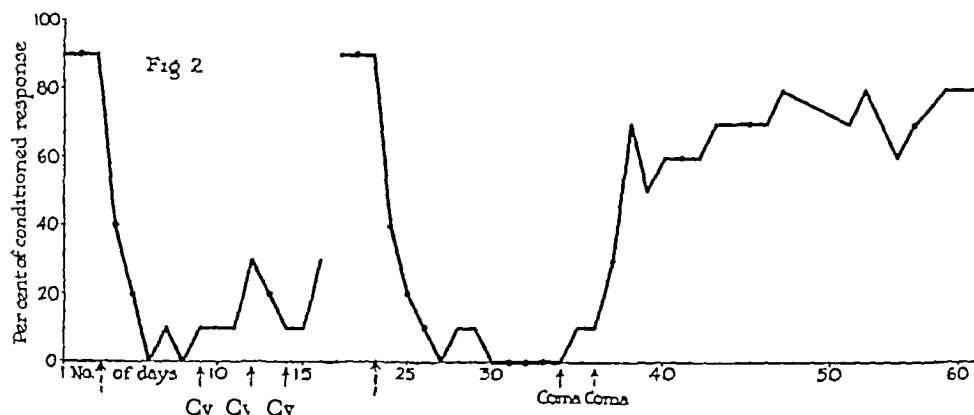


FIG 2 Comparison of the action of insulin convulsions with that of coma General arrangement as in Fig 1 At the arrows 10 units of insulin per/kg were given which resulted in convulsions on the 9th, 12th and 14th days Insulin convulsions were ineffective The rat was then reconditioned and hereafter inhibited Two comas (34th and 36th day) led to a permanent restoration of the conditioned response Cv in figure stands for convulsion

on repetition By properly timing the coma it seems to be possible to permanently restore the inhibited c r

The first graph of Fig 2 shows that 3 insulin convulsions did not sig-

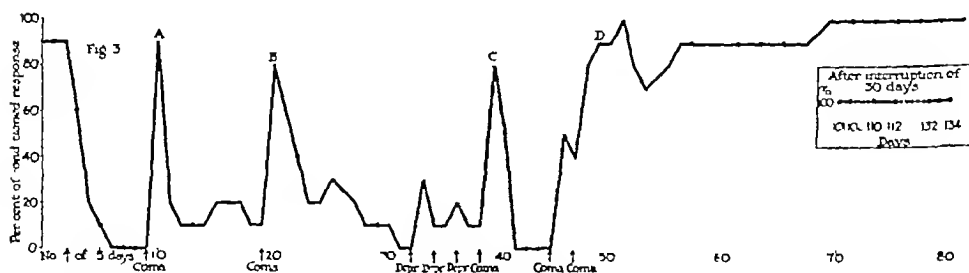


FIG 3 General arrangement as in Fig 1 Insulin "depressions" were ineffective whereas even a single coma caused temporary recovery (A, B, C) Permanent recovery was affected by two comas induced on the 44th and 46th day

nificantly alter the state of inhibition The animal was then reconditioned and reinhibited (second graph of Fig 2) The rat was again subjected to insulin The first insulin injection caused slight convulsions and was not effective, the second induced a coma and was followed by a prompt recovery which lasted for 22 days until the death of the animal

extremities, slow righting reflexes and salivation. At this stage the rat reacts to pain but not to slight pressure, (ii) insulin coma in which righting reflexes and reactions to painful stimuli are abolished, (iii) tonic-clonic hypoglycemic convulsions.

Ordinarily, these stages followed each other in brief intervals. Occasionally, however, convulsions occurred very suddenly without having been preceded by a comatose phase.

The convulsive phase had to be terminated immediately since an attempt to keep the animal in this condition resulted in death in most in-

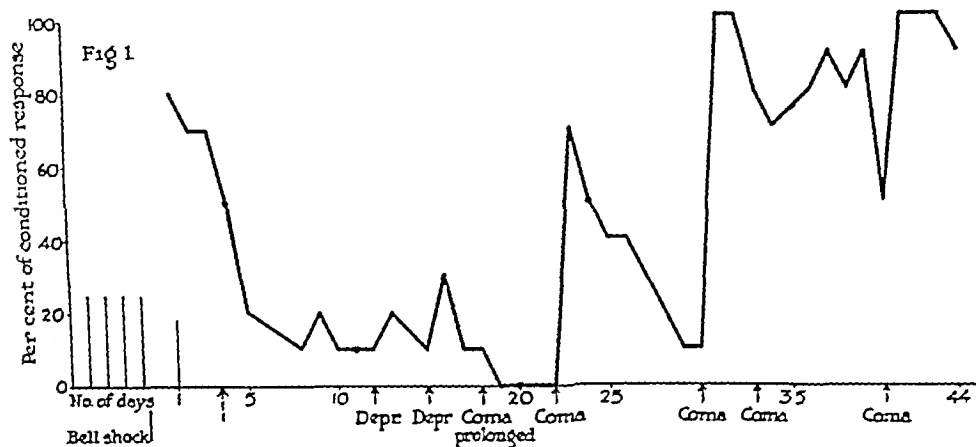


FIG 1 Effect of insulin hypoglycemia on the restoration of previously inhibited conditioned reactions. Vertical lines at the beginning of the graph show the number of reinforced conditioned stimuli (bell plus shock) which established the conditioned response. It was maintained for three days at 70 to 80 per cent and then inhibited by lack of reinforcement. On the 12th day 4 units/kg of insulin were given intraperitoneally, on the other days marked by an arrow 5 units/kg were administered. The experiment showed that hypoglycemic "depressions" were unable to restore the conditioned reaction but coma caused recovery. Note the cumulative effect of three comas given between the 30th and 40th day.

stances. The coma was allowed to persist for about 7 minutes since after this period convulsions ensued frequently. The period of depression was of similar duration.

In Fig. 1 it is shown that the injection of insulin leading to a depression on the first two occasions did not alter significantly the c r of the inhibited rat. Several days later insulin was again injected and resulted in a deep coma which was without effect on the c r. However, 4 days later when the injection of insulin resulted again in coma the c r recovered up to 70 per cent. When 8 days later the c r had returned to the level of 10 per cent, 3 more comas were produced by insulin, the second followed the first after 3 days and the third followed the second after 7 days. The results show very strikingly a recovery of the c r to 100 per cent which is only slightly diminished some days later because the subsequent insulin comas tend to restore the

cent in spite of the complete lack of reenforcing stimuli. The period of testing was interrupted for one month without altering this record.

In two more animals a complete recovery was observed for 80 and 90 days respectively at which time the experiment was discontinued. In another animal repeated insulin injections led to a restitution of the inhibited reflexes for more than 30 days and then a gradual decline occurred.

The experiments prove conclusively that by properly spaced insulin comas inhibited conditioned reflexes can be permanently restored. Moreover, this effect continues in spite of the fact that the "permanently recovered" rats do not at any time receive the reenforced c s. They jumped from one

*Table 1 Specificity of the recovery of the conditioned response after insulin coma\**

Rats	Application of 5 Sound Stimuli (A) from Oscillator Followed by 10 Bell Stimuli (B)			Application of 5 Stimuli B (bell), followed by 3 Sound Stimuli A, followed by 5 Stimuli B, followed by 2 Stimuli A		
	Days	No. of Resp to Sound A	No. of Resp to Bell B	Days	No. of Resp to Sound A	No. of Resp to Bell B
11	1	0	10	1	0	10
	2	0	9	2	0	10
	3	1	10	3	1	10
13	1	0	10	1	0	10
	2	0	10	2	0	10
	3	0	10	3	0	10
14	1	1	10	1	0	10
	2	0	9	2	1	10
	3	0	10	3	0	10
15	1	0	8	1	0	10
	2	1	10	2	0	9
	3	0	10	3	0	9

\* All rats had been previously conditioned only to the "bell" and not to the sound of 250 vibrations produced by an oscillator.

compartment into the other before the shock which was customarily applied after an interval of two seconds could become effective.

The observation that rats in which the c r had been inhibited show a restitution of this response either temporarily or permanently following insulin treatment raises the question whether this effect is due to a disinhibition of these animals or is simply the result of a generally increased excitability which induces the animal to jump in response to the bell. Two series of experiments were performed to decide this question. In the first, 3 normal rats which had not been previously conditioned were subjected to two insulin comas on alternate days and tested daily for 15 days following insulin coma. The results showed that although all these animals were tested in exactly

In the experiment reproduced in Fig 3 the sequence of insulin depression and coma was reversed and under these conditions hypoglycemic depression failed likewise to restore the inhibited c r. In this animal a single coma was sufficient to produce a marked but temporary recovery. This effect was repeated twice before the effect of hypoglycemic depression was studied. Although each insulin coma brought about a recovery of 80 or 90 per cent, even 3 insulin depressions failed to alter the c r significantly. A coma administered subsequently caused an effect similar to those obtained earlier in the same animal. The experiments show that insulin hypoglycemia leading to depression only was completely ineffective in this animal.

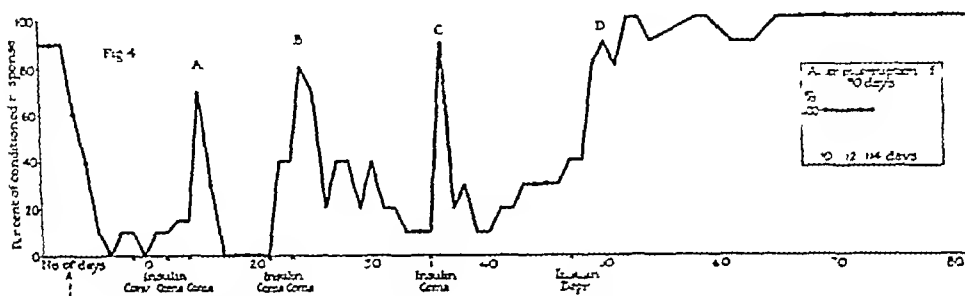


FIG 4 Temporary and permanent effects induced by 10 units of insulin per kg at the arrows

After the effect of the third coma (C) had passed off insulin was injected on two alternative days leading in each case to coma. The effect was very striking. Not only did it lead to a recovery of the inhibited c r but this effect was also maintained for about 3 months. During this time the animal was frequently tested with 10 non-reinforced c s which were *not* preceded by the customary reinforced stimuli. But even this procedure failed to induce any inhibition in this animal.

A similar case of permanent recovery of previously inhibited c r is illustrated in Fig 4. There are, however, individual features in this experiment which are worthy of mention. The first insulin injection led to convulsions and was without effect. The second and third insulin injections produced coma which led to a temporary recovery for two days only. After this effect had completely disappeared, two more insulin comas were given which resulted in a recovery of distinctly greater duration. This cumulative effect may be responsible for the fact that a single coma given later (C) produced an effect at least as great as the first two comas had elicited. In addition, it was found that there was a gradual increase in the number of positive c r occurring between the 8th and 13th day after the last coma. At that time the animal was very alert and was again subjected to insulin which led only to a depression. However, this "treatment" was followed by a marked recovery which proved to be permanent. The animal was observed for more than two months following the last insulin injection. C r were maintained at 100 per

are therefore not utilized for averaging the results Table 2 shows that when 20 reenforced stimuli are given following the injection of saline, the conditioned response was on the average 20 per cent, the individual values varying between 10 and 20 per cent The experimental group injected with insulin was given on the average only 15 reenforced c s , but in spite of this lesser

Table 2 Effect of Insulin Hypoglycemia on Partially Conditioned Rats

A Controls

Animals	No of Bell + Shock Applied for Partial Conditioning	Amount Saline Injected	No of Bell + Shock Applied after 2nd Saline Inj	Total No Bell + Shock Applied	Conditioned Response on Testing
1	95	0 5 cc	20	115	40 %
2	77	0 5 cc	20	97	30 %
3	50	0 5 cc	20	70	10 %
4	53	0 5 cc	20	73	20 %
5	81	0 5 cc	20	101	40 %
6	110	0 5 cc	20	130	0 %
7	55	0 5 cc	20	75	20 %
8	31	0 5 cc	3	34	90 %*
9	82	0 5 cc	20	102	0 %
Average	75		20	95	20 %

B Insulin-Injected Rats

Animals	No of Bell + Shock Applied for Partial Conditioning	Amount Insulin Injected in u/kilo Wt	Effect of First Insulin Injection	Effect of Second Insulin Injection	No of Bell + Shock Applied after Insulin Coma	Total No of Bell + Shock Applied	Conditioned Response on Testing Per cent
1	22	10 u/kilo	Coma	Coma	7	29	100*
2	70	10 u/kilo	Depression	Coma	20	90	100
3	55	10 u/kilo	Coma	Coma	10	65	90
4	83	10 u/kilo	Coma	Coma	15	98	100
5	82	5 u/kilo	Depression	Coma	20	102	80
6	115	5 u/kilo	Depression	Coma	15	130	100
7	95	10 u/kilo	Depression	Coma	20	115	0
8	50	4 u/kilo	Coma	Coma	10	60	100
9	51	4 u/kilo	Coma	Coma	10	61	90
Average	75				15	90	82 5

\* Not included in the average

degree of training the c r amounted to 82 5 per cent There was only one animal in the experimental group in which the conditioned response was not established

It was mentioned earlier that we had in each group a rat which could be conditioned more easily than most animals and it was therefore, decided to exclude these animals from the average Both rats showed clearly complete

the same manner as the rats described in the preceding section, they did not react at all to the sound of the bell. These results, therefore, are conclusive in showing that animals which never have been conditioned before do not spontaneously respond to the sound of the bell even though they have been subjected to insulin coma. Apparently, the insulin "treatment" can become effective only after the c r has been previously induced and then inhibited.

In a second group of experiments the question was investigated whether the restitution of the c r was specific for the previously inhibited c s or whether a positive response would result from the application of other stimuli as well. The experiments of Table 1 show that when insulin coma had resulted in a complete recovery of the previously inhibited c r to the bell almost no positive response was obtained when a sound of about 250 vibrations (S-250) was substituted for the bell. Experiments on 3 more animals, not included in Table 1, showed a similar result. In two of them the animals had been inhibited to the bell and showed restitution of the c r after insulin. No reaction to S-250 occurred. In a third experiment in which the c r to bell and to S-250 had been inhibited the response was restored to both c s by insulin. It was found also that a light which was not previously used as c s was completely ineffective although in other experiments in which the rat had been conditioned to light this reaction could be inhibited and then restored by insulin. It may, therefore, be concluded that insulin coma results in disinhibition of previously inhibited conditioned reflexes.

In view of the results described in the preceding paragraphs the important question arises whether the action of insulin coma on conditioned reflexes consists only in the removal of inhibitions, or whether under the influence of insulin coma excitatory reactions which are the basis of conditioning processes are likewise influenced. In order to decide this question a group of experiments was performed on the effect of insulin coma on partially conditioned rats. Two groups of 9 rats were chosen for this experiment. Whereas in the main experiments described previously the rats were conditioned until they responded with the c r in nearly 100 per cent to the c s the "partially" conditioned rats were trained only up to the time when their behavior indicated that the conditioning process had begun. After various trials the following procedure was chosen. The rats were subjected for 2 to 5 days to not more than 25 reinforced c s (bell+shock) on each day. As soon as the animals jumped twice in succession in response to the c s before they were stimulated by the u s the training period was terminated. This occurred on the average, after 75 reinforced c s had been applied. Then the rats of one group were injected with 0.5 cc saline on alternate days and rats of the experimental group were given insulin on two alternate days. On the following day 20 bell+shock stimuli were applied and then 10 non-reinforced c s were given in order to ascertain the state of conditioning. In each of the two groups there was one animal ( $\pm 8$  of the control and  $\pm 1$  of the experimental group) in which the conditioning reaction was established with an unusually small number of combinations of c s and u s. These animals

However, the chronic effect of insulin hypoglycemia on the brain is not restricted to the elimination of intracentral inhibitions of the Pavlovian type. The experiments on partially conditioned rats show also that the excitatory processes which form the basis for the establishment of c r are facilitated. This is shown by the fact that insulin administered during the training period greatly enhances the formation of c r. It is of interest to

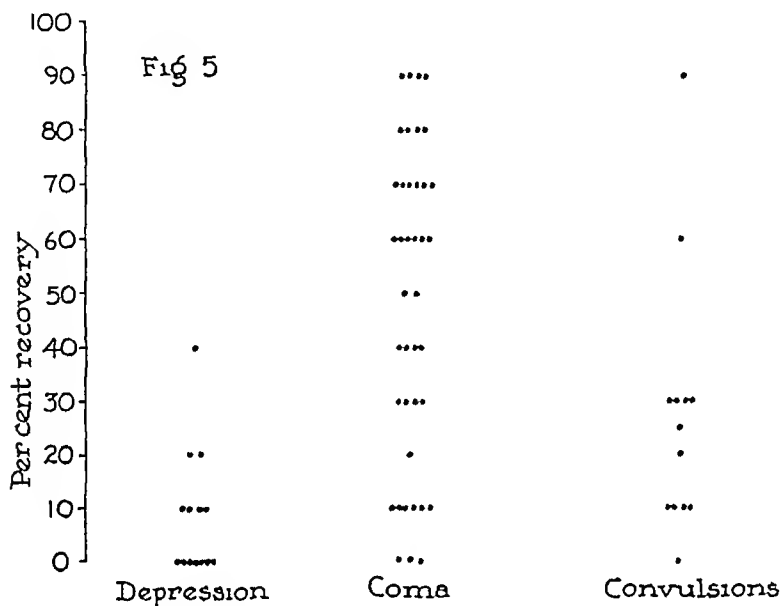


FIG 5 Relative efficiency of insulin "depression," coma, and convulsions on the restitution of previously inhibited conditioned responses. The ordinate refers to the increase in the percentage of the conditioned response following administration of insulin.

point out that the chronic effects of insulin hypoglycemia are similar to those described by Kessler and Gellhorn (4) for metrazol and electroshock. Whether it is possible to induce a permanent recovery with these last two procedures has not yet been investigated, nor is it known whether these convulsive procedures enhance the *formation* of c r as has been shown for insulin in the present investigation.

The data presented in this paper as well as in the investigations of Kessler and Gellhorn (4) suggest that central sympathetic excitation which is common to all three procedures (1, 2, 3) may play an important part in the explanation of the altered behavior of man and animals subjected to various forms of "shock therapy." It will be the task of further studies to determine the neural structures which are necessary for the disinhibition of conditioned reactions by means of insulin coma or experimentally induced convulsions.

conditioning in the control as well as in the experimental group. The fact that under control conditions an average of 95 combinations of c s and u s led in 8 animals to c r of only 20 per cent, whereas, 90 combined u s and c s led in the same number of animals to a c r in 82.5 per cent when the animals had been subjected to two insulin hypoglycemias shows clearly that the chronic effect of insulin hypoglycemia is not restricted to its action on intracerebral inhibition but affects likewise those excitatory processes which are the basis of the establishment of c r.

### DISCUSSION

In 1938 Rose, Tanton-Pottberg and Anderson (5) published a report on a single sheep in which after a conditioned reflex had been lost spontaneously this reflex was restored following a series of insulin shocks. The present report is a confirmation and extension of this work. It has been shown in normal rats that permanently inhibited conditioned reactions may be restored by means of insulin hypoglycemia. The effect of insulin was cumulative in as much as two to three comas were almost regularly effective whereas one coma frequently failed to alter the inhibited conditioned reactions. The duration of the effect was variable. Properly timed insulin comas resulted in a permanent recovery of the inhibited conditioned reactions. The behavior of the animals thus influenced was altered. They seemed to be more alert than prior to the "treatment." Moreover, the application of the non-reinforced c s to these "permanently recovered" animals failed to induce any inhibition as it regularly did in all animals not subjected to insulin hypoglycemia. When insulin coma failed to effect a permanent recovery of the c r a temporary restitution of the c r lasting several days was observed. It will be of interest to determine the factors underlying these individual differences.

It was mentioned earlier that the clinical syndrome resulting from the administration of insulin may be classified as depression, coma, or convulsion. Figure 5 gives a survey of all the results thus far obtained. It shows clearly that insulin coma is very effective whereas convulsions only infrequently restore inhibited c r. As to hypoglycemic depression it may be said that it is mostly ineffective. One instance in which a significant increase in c r could be observed is that seen in Fig. 4 in which the c r were already partially disinhibited as a result of previously administered insulin comas. Such an effect has been observed once more subsequently under similar conditions.

The fact that insulin coma restored inhibited c s was interpreted as being due to disinhibition of c r. This interpretation is justified on the basis of two sets of experiments. In the first it was shown that insulin coma failed to cause any measurable effects in rats which had not been conditioned previously, secondly, the reaction was specific in as much as the rats showed a positive response to the bell to which they had previously been conditioned, but failed to react to other sounds or visual stimuli.





## SUMMARY AND CONCLUSION

The chronic effect of insulin hypoglycemia on the central nervous system has been studied by means of conditioned reactions

Normal rats which jump from a compartment A across a small partition to a compartment B in a response to an electric shock applied to the grid of the compartment (unconditioned response) are trained to react in a similar way in response to a conditioned stimulus (bell) This reaction is inhibited by lack of reinforcement Under such conditions no spontaneous recovery occurs but insulin hypoglycemia restores the inhibited conditioned response The action of insulin is cumulative and may lead to a permanent recovery This effect of insulin depends upon the number of insulin administrations and the degree of hypoglycemia, coma being the most effective procedure The reaction is specific since no positive responses are elicited when stimuli are used to which the animal had not been conditioned previously

The chronic effects of insulin coma are not restricted to disinhibition of conditioned responses but influence also the excitatory processes which are the basis of conditioning This is shown by the fact that a partial conditioning leading to an average of only 20 per cent positive responses in the control group causes 82 per cent conditioned responses in the experimental group subjected to two insulin hypoglycemias during the training period

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placed at the start again. This 15-second interval insured that the recent memory being tested was of at least this duration. Eleven trials each day were given in order that ten opportunities to alternate be provided. Preoperative tests consisted of six such days, thus allowing a maximum of 60 alternations. At the end of this time the scores of all animals were examined and those animals were discarded which had not alternated 36 or more times, that is, 60 per cent of the possible number, and that percentage which is significantly different, statistically, from 50 per cent. Six of the 24 animals did not reach this criterion and were dropped from further study. The remaining 18 were then subjected to operation.

The operative technique was a modification of the method of thermocoagulation previously described by Finley (4). The operative instrument was an electric soldering iron whose tip was cut down to present a round flat surface 3 mm in diameter. This tip was applied to the surface of the skull bones after an incision had been made, under ether anesthesia, in the skin and cranial musculature. By varying the position on the top of the skull and by regulating the time and temperature of the application according to calibrations previously worked out on trial animals, lesions could be made in the cortex in almost any desired position and of an appropriate size. The resulting lesions were almost perfectly circular in shape, were clearly demarcated from surrounding intact tissue, and were free from the mechanical distortion which often accompanies the use of a thermocautery applied directly to the cortex. In addition to these features, the technique has the advantage of producing much less operative shock than methods requiring trephining of the skull.

The animals were kept upon the 24-hour feeding schedule over the operative and recovery period so that there would be no changes in motivation to disturb postoperative testing. This was begun 7 to 10 days after the operation, and like the preoperative tests, was conducted for 6 days at 11 trials per day. In a few instances animals were run 21 trials per day, thus allowing 20, rather than 10, alternations, but analysis of the records shows that this had no effect upon the final results. One animal died three days after operation and thus did not enter upon postoperative tests.

Upon conclusion of these tests, animals were kept alive for one month or more after the operation before removing their brains. This was done immediately upon death under ether and the brains were placed in 95 per cent alcohol for two weeks. After fixing and before blocking, the position and extent of the lesions were determined in each case by using dividers and drawing the lesions upon standard diagrams prepared by Lashley (11). In view of the fact that the operative technique produced cleanly demarcated lesions without distortion in the shape of the brain, this method of reconstruction was just as accurate as that done from histological sections, except that damage to subcortical nuclei could not be seen in this way. Previous studies of the effects of the time and temperature of the thermocoagulation, however, had already indicated that no subcortical damage, except in case of infection, was to be expected.

## RESULTS

The pre- and postoperative performances on the delayed alternation tests, as well as a specification of the position and extent of the lesions in each case, are summarized in Table 1. In order to make the results readily understood, the rats have been arranged and numbered approximately in the order they were affected by the operation. Rats 1 and 2, for example, were most affected and rats 16 and 17 least affected by the operation.

Table 1 gives the pre- and postoperative performance of each rat and Fig. 1 shows the position and extent of each lesion as ascertained after removal and fixing of the brain. It will be seen in Table 1 that there was some falling off in postoperative performance of every single animal irrespective of the kind of cortical lesion inflicted. In many cases the reduction was slight, yet there is no exception to the rule. This fact may indicate one of two conclusions: either that there is a general tendency for spontaneous alternation to decrease as animals have run more and more trials or that a cortical injury of any kind reduces somewhat the amount of alternation.

# CORTICAL LOCALIZATION OF SYMBOLIC PROCESSES IN THE RAT II. EFFECT OF CORTICAL LESIONS UPON DELAYED ALTERNATION IN THE RAT

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DESPITE THE fact that the memory of maze performance in rats is not dependent upon any specific areas, recent experiments by Stellar, Morgan and Yarosh (20) have shown that a maze-performance, which excludes the use of external cues and requires symbolic processes, depends upon the anterior cortical areas remaining intact. This finding parallels the special role of the prefrontal areas of primates in controlling the mechanisms of recent memory and the synthesizing of a series of acts (9), and indicates that in the rat, as in primates, there may be specialized "association" areas.

As a further attack upon this problem we have selected another behavioral test, the delayed alternation, which requires the use of immediate memory, and have determined how it is affected by the removal of various cortical areas. An early study by Loucks (13) made use of a similar test and indicated that lesions of the "prefrontal" areas abolished ability to pass it, but his experiments failed to provide sufficient assurance that the same results might not also be obtained by lesions in other areas, and for this reason, as well as for verification, it seemed profitable to reinvestigate the problem.

## METHODS

A number of years ago Hunter (8) devised the delayed reaction test of recent memory and the rat's performance on the test was subsequently studied in several experiments (16). A large number of trials was required to learn it, however, and at best rats never did very well, these two factors tended to discourage the use of the test in operative studies.

Recently Heathers (7) and others (2, 3) have described a phenomenon of spontaneous single alternation in rats, which involves the use of recent memory and serves the purpose of the present study. Rats are simply run on a T-maze in which both choices are rewarded, and once they have become familiar with the apparatus, they begin spontaneously to alternate, taking first the left alley, then the right, then the left, and so on. There has been considerable discussion as to why rats do this, but that need not concern us. The fact is that they do, and that in order to alternate at higher than a chance level they must remember what turn they took on the previous trial. Thus immediate memory, and not any habit learned over a series of trials, must be the basis of the alternation.

In the present study a T-maze of the following specifications was used: elevation, 30 inches above the floor, width of path, 2 inches, length of starting arm, 24 inches, length of each side arm, 18 inches. Purina dog chow, mixed with water to make a mash, was present at the ends of both arms at all times.

Twenty-four brown rats were begun in the experiment. They were first put on a 24-hour feeding schedule and allowed enough food (Purina mash) in each feeding period to bring them down in weight to a point where they would be healthy but highly motivated. This schedule was maintained for a week. Then on the eighth day they were placed, two at a time, on the maze and allowed to explore and eat for a half-hour. Finally, on the ninth day, regular tests of recent memory were run. They were placed on the start and allowed, without any forcing or interference, to choose either arm of the T to get food. They were allowed 15 seconds of eating, clocked on a stop watch, before being taken from the arm and

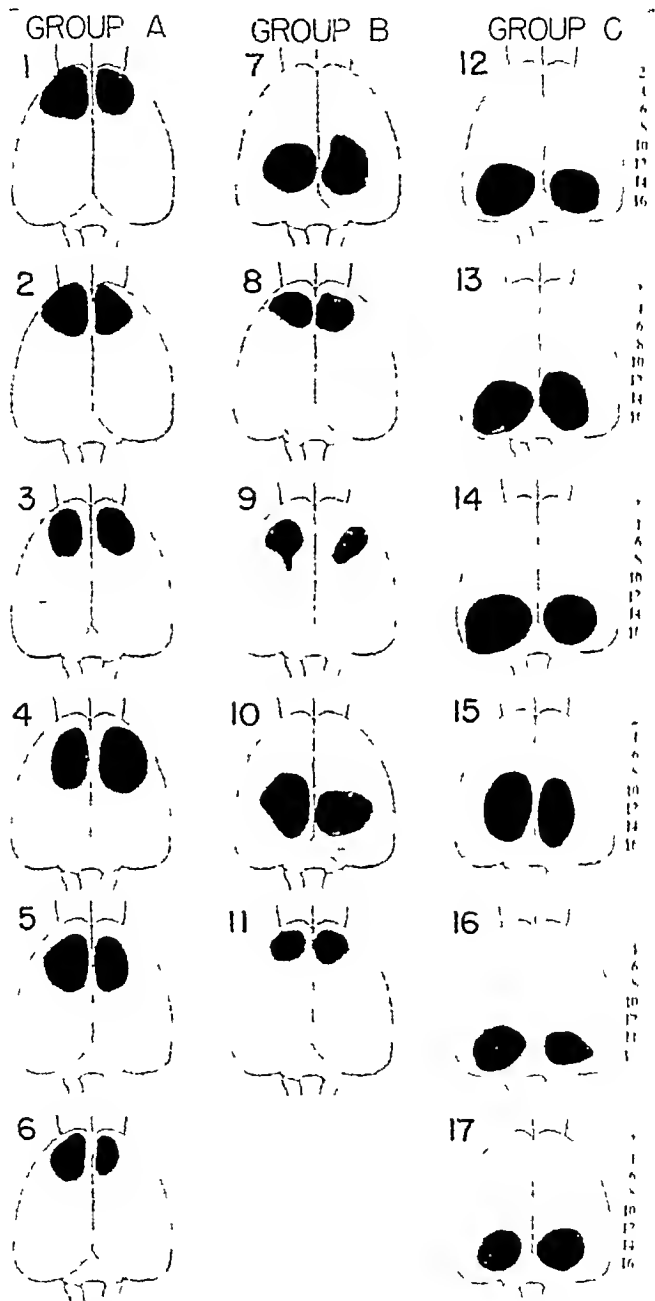


FIG 1 Maps of the lesions sustained by the 17 rats tested on delayed alternation. Group A (Nos 1-6) consists of animals whose behavior was greatly affected by operation, Group B (Nos 7-11) of animals with small or doubtful postoperative disturbances, and Group C (Nos 12-17) of rats not affected significantly by operation.

Experimental evidence available at the present time does not allow us to decide definitely between these possibilities, although there is some indication (7) that single alternation tends to decline somewhat with time

Since the purpose of this study is to determine whether or not capacity for single alternation is "localized," the over-all decrement in performance is important only because some allowance should be made for it in considering how seriously operations affected the alternation. Taking this tendency into consideration, the cases may be divided into three groups. Group A, consisting of 6 animals (Nos 1-6), in which alternation was almost com-

*Table 1 Percentage of single alternation in 60 preoperative and 60 postoperative trials*

GROUP A Prefrontal Lesions			GROUP B Various Lesions			GROUP C Occipital Lesions		
Rat No	Pre-	Post-	Rat No	Pre-	Post-	Rat No	Pre-	Post-
1	80	10	7	75	25	12	67	57
2	83	13	8	70	28	13	70	60
3	62	0	9	70	41	14	75	65
4	77	15	10	78	55	15	68	62
5	68	10	11	82	70	16	62	58
6	77	19				17	62	58

pletely abolished and was, in any case, reduced below the 50 per cent level expected of animals making their choices completely at random, Group B, made up of 5 animals (Nos 7-11), in which the operation was of less consequence, and Group C, comprising 6 animals (Nos 12-17), whose post-operative performance was not significantly reduced. These may be designated the affected (A), doubtful (B), and unaffected (C) groups respectively.

For purposes of exposition, we may divide the rat's cortex into four general areas, delimited by the numbered levels on the prepared diagrams, and assign them names comparable to roughly corresponding areas in the primates: prefrontal areas, levels 1 to 4, frontal areas, levels 4 to 9, parietal areas, levels 9 to 13, and occipital areas, levels 13 to 18.

The lesions sustained by animals of Group A are shown in the left hand column of Fig 1. In every single case, it is to be noted, the prefrontal quarter of the cortex is significantly implicated. In the two most affected cases, the lesion is squarely at the anterior poles. In three other less affected cases, some of the more posterior frontal ("motor") areas are involved, but all of them overlap the prefrontal areas somewhat.

The unaffected animals in Group C all have lesions in the posterior cortex, in most cases overlapping the visual areas, and safely out of the central dorsal region subserving motor functions. They present positive proof that the occipital areas are not necessary to the functions involved in spontaneous single alternation.

distance-discrimination test of symbolic processes was studied, it was pointed out that the critical areas for symbolic functions in the rat, although not well defined, tend to lie in the fields of projection of the anterior and medial divisions of the ventral thalamic nucleus. The same statement can be made from the present findings. It should be noted, however, that the projections of the dorsomedial thalamic nucleus also project to this region of the cortex (11). In primates this nucleus projects to areas 9, 10 and 12 of the prefrontal lobe (21) and degeneration in it is associated with the psychological phenomena of prefrontal lobotomy in human individuals (5). It is logical, therefore, to suppose that the effects upon recent memory which are here reported depend upon the projection field of the dorsomedial nucleus. There are, unfortunately, no comprehensive studies of the exact areas served in the rat by this nucleus, but only observations based upon a few cases (11). Taking these observations, however, and also the result of this and the previous study (20), there is now good reason to believe that there exists in the rat a prefrontal area which is roughly comparable anatomically and in the behavioral functions which it serves to the prefrontal "association" areas of man.

Lashley's earlier studies (12) of cortical areas involved in maze learning yielded no evidences of functional localization in the rat's cortex. The number of psychological elements concerned in maze learning, however, is so great that this might be true despite relatively discrete functional localization of different specific capacities. Studies, moreover, in which less complex behavioral performances have been considered, have tended on the whole to indicate a limited localization of functions. The striate area is necessary for visual pattern discriminations (10), a temporal area for certain auditory discriminations (6, 17, 22), a dorsal "motor" area for hopping and placing reactions (1), a more frontal area for motor coordination (14) and for hand preference (18), and another dorsolateral area for tactual discrimination (19). Now, to this list should be added a prefrontal "association" area for recent memory.

It may turn out upon further study that the loss following removal of this prefrontal area is not so much of recent memory as it is of the ability to maintain a set in the face of distraction. That at any rate has been the case in the primate studies. Prefrontal lobectomized monkeys are capable of solving delayed reaction tests if precautions are taken to see that the animal is not distracted during the delay (15), whereas under ordinary circumstances the capacity is abolished by prefrontal lobectomy (9). In our experiments animals were open to considerable distraction during the delay, they were picked up from the feeding dish, moved to a chair where they remained during the delay while the experimenter was moving around and recording the results of the previous trial, and then finally picked up and set down on the start again. Thus our experiments might very well find recent memory disturbed by the operations when the more fundamental factor is ability to withstand distracting influences. In further experiments the attempt can be made to separate these two factors from each other.

The picture presented by Groups A and C is perfectly clear and consistent, and it calls for the "localization" of the capacity of delayed single alternation in the anterior cortical areas. Group B, the doubtful animals, presents some difficulties, however. One case in it, No. 8, falls in line with the other results, for it shows a postoperative decrement which is probably significant and its lesion is largely prefrontal. Case No. 9, however, has little involvement of the prefrontal region, yet it shows a moderate, but significant, postoperative loss. From it we might suppose that the frontal motor areas also function in recent memory. Case No. 7, on the other hand, shows a loss almost as great as those in Group A, and its lesion is in the parietal areas. Case No. 10 is like it in respect of position of lesion, but the loss in behavioral performance which it sustained, though probably significant, is less definite. One animal in Group C (No. 15), on the other hand, has almost the same type of lesion as Nos. 7 and 10, and it shows no postoperative deficit. Thus, the parietal lesions do not give consistent behavioral effects.

Case No. 11, in Group B, is the last to be considered. It shows hardly any postoperative loss, but its lesion is prefrontal. It is, in fact, the only one of 9 cases with prefrontal involvement that was not significantly impaired by the operation. It may be pointed out, however, that the size of its lesion was small compared to the other prefrontal cases, and an inspection of the depth of the lesion, in addition, indicates that it probably does not penetrate the entire cortex. Assuming, then, that a reasonably large area of the prefrontal cortex is involved in the recent memory function, it might be said that this case left enough of the critical area intact to allow it to function without significant impairment.

### DISCUSSION

Making allowances for the superficial lesion of case No. 11, two definite conclusions can be drawn from the present results: destruction of the prefrontal areas abolishes passable performance in the delayed alternation test, and lesions of the occipital areas have no significant effect upon such performance.

The role, however, of the "motor" and parietal areas in this performance is left in doubt. The one case of a motor lesion free of implication of the anterior areas showed a similar, though smaller, reduction in score. Parietal lesions, on the other hand, did not give a consistent picture, one produced considerable impairment, another slight impairment, and still a third none at all. In view of the motor and kinesthesia functions subserved by these areas between the anterior and occipital poles, it is probably to be expected that their extirpation might have some effect upon the performance, but the present results do not indicate that such a possible role is primary. It is probably best to conclude, pending future experimentation, that the parietal areas do not make a primary contribution to the performance but may have some importance in it.

In the previous paper by Stellar, Morgan and Yarosh (20) in which the



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## SUMMARY

The experiment was designed to determine whether or not there are limited areas in the anterior cortex of the rat which subserve symbolic processes, and in particular recent memory, as has been found to be the case in primates

In a T-maze in which rats spontaneously alternate, 17 rats were run for 60 trials each, pre- and postoperatively. Between each trial there was an interval of 15 seconds and single alternation significantly greater than a chance level of 50 per cent was taken as an index of the use of recent memory. Following completion of postoperative tests brains were removed and the size and location of the lesions reconstructed upon standard diagrams. The majority of the lesions were in either the prefrontal or the occipital areas. Animals with the prefrontal lesions, except for one with a small lesion, lost their ability to use recent memory, whereas those with occipital lesions showed little difference in performance. Too few 'pure' motor lesions were available to determine whether these also affected the recent memory. Parietal lesions, on the other hand, gave inconsistent results.

Some "localization" of recent memory in the anterior areas of the rat is definitely indicated, although more work is required to tell us how precise it may be. Considering the other cases of functional localization of specific functions established in recent years, the rat's cortex appears not to be so poorly differentiated as earlier maze studies lead us to believe.

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## EXPERIMENTAL DATA

A summary of the findings follows. The results of stimulation of cerebral cortices are prefaced by a brief description of the motor status of each animal just prior to the terminal experiment.

*Experiment 1 (R S 8) July 29, 1942* A large, nearly mature male, wt 4.2 kg Lt areas 4 and 6 removed Feb 20, 1942

During the five months since the first operation the right motor paresis had become slight and had not improved after the first two or three months. The paresis was characteristic of that seen in all animals following similar lesions. There was weakness and awkwardness in the right extremities particularly in the hand. However, the animal was able to

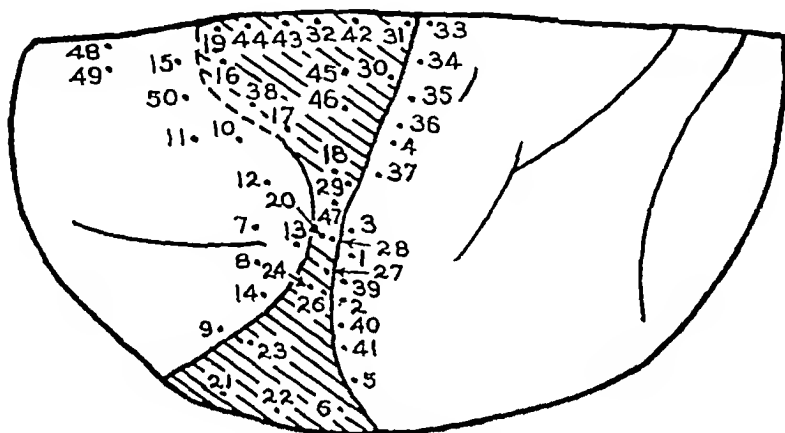


FIG 1

climb using all four extremities and prehension was present in the right fingers although of a simple order as compared to that of the normal left hand. Tendon reflexes were increased on the right side, there was slight atrophy of the limb muscles of this side and slightly increased resistance to passive manipulation.

*Stimulation* In Fig 1, the points stimulated on and about the scar of the left areas 4 and 6 ablation are designated numerically. No response to any stimulus was obtained from any point except from the following

Point	Response
7	Dilatation of lt pupil
8	Eyes turned rt
9	Convergence of eyes
10	Lid blinking, convergence
12	Blinking followed by pupil dil and lachrymation from rt eye
13	Rt eye turned up, lt down, long latency
38	Eyes turned rt, blinking, movement of rt arm
48	Blinking
49	Blinking
50	Eyes turned rt arm movement

*Summary* Stimulation elicited appropriate movements of eyes, pupils and lids from area 8. Diffuse arm movements were also obtained from what was thought to be area 8 but may have been borderline area 6. The entire postcentral gyrus and the scar tissue were inexcitable.

*Experiment 2 (I 37) Jan 19, 1942* A male, 3 years, weight 2.4 kg Born Apr 27, 1939,

# MOTOR RESPONSE TO STIMULATION OF CEREBRAL CORTEX IN ABSENCE OF AREAS 4 AND 6 (*MACACA MULATTA*)\*

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PREVIOUS experiments have established two facts with regard to the influence of the cerebral cortex on motor performance of monkeys (1) Ablation of the motor areas (areas 4 and 6 of Brodmann) in infancy has less effect than removal of the same areas from older animals, (2) removal of the frontal areas (areas 8-12) or of the postcentral gyrus (areas 3, 1, 2) from normal animals does not cause paresis, but, if areas 4 and 6 have been previously removed in infancy, subsequent extirpation of either frontal or post-central regions is followed by a marked increase in motor deficit (2)

Some reorganization of function of these "non-motor" regions must thus occur after areas 4 and 6 have been removed in infancy Since these altered areas might then become more excitable to cortical stimulation, the cortices of five nearly adult monkeys have herewith been stimulated following motor ablations made at a much earlier date Four of these animals had been operated upon in infancy and the excitability of their cortices explored more than two years thereafter The fifth, used as control, was nearly mature and had been operated upon five months previously. In all, apparently maximal recovery of function had taken place some time before the terminal experiment

## METHOD

The five animals were all *Macaca mulatta* The four operated upon in infancy were born in the colony and of known age The fifth was much older, a nearly mature male weighing 4.2 kg All cortical ablations were made by the same individual and with the same technique Areas 4 and 6 were totally removed from one or both hemispheres of four monkeys The lesion extended to the depth of the cingulate gyrus on the medial aspect and through the face area laterally It is probable that all of area 6b was not removed in every case, since its margins are indefinite and vary with the shape of the arcuate sulcus The extirpation was taken to the depth of the central sulcus The fifth animal had bilateral extirpation of areas 4, 3-1-2 comprising the pre- and postcentral gyri Area 6 remained intact

It was necessary in the case of the control animal to use a preparation with a unilateral ablation because older animals with bilateral ablations are so severely paralyzed that they are difficult to keep alive, and, it was felt that a preparation of long-standing to insure maximal recovery was essential for comparison with those of infants showing maximal recovery after bilateral ablations At the time of sacrifice, the cortices were exposed under dial anesthesia (0.6 cc per kg given one-half intraperitoneally and one-half intramuscularly) Stimulation was with bipolar electrodes by a current controlled for wave form, frequency and voltage After removal of brain at autopsy, sections were made and stained by Nissl technique so that the extent of the lesions might be verified histologically

\* Aided by grants from the Friedsam Foundation (Child Neurology Research), and the Macy Foundation

ages Higher voltages caused the same movement at longer latency from any spot in the entire scar This was obviously due to spread of current via the scar tissue Movements of arm and face were elicited from the entire length of the posterior lip of the central sulcus

*Experiment 3 (I 50) July 17, 1942 A male, 2 years, weight 2.1 kg Born Jan 8, 1940 Bilateral ablation of areas 4 and 6 Jan 30, 1940*

Simultaneous bilateral removal of areas 4 and 6 produced a deficit in this infant which was similar in kind but slightly more severe than that of the animal in Expt 2 There was marked crossing of the hind limbs in walking, increased resistance to passive manipulation and active tendon reflexes Voluntary prehension was almost impossible, but climbing and clinging were well executed

*Stimulation* In Fig 3, A and B, the points stimulated on the left and right cortex respectively are indicated numerically No response was obtained except from the following points

<i>Lt Cortex</i>		<i>Rt Cortex</i>	
<i>Point</i>	<i>Response</i>	<i>Point</i>	<i>Response</i>
5	Arm, wrist extension	1	Face
7	Arm, more marked	2	Face
8	Elbow	3	Face
9	Diffuse arm	5	Face
10	Elbow and wrist, eversion	6	Face
11	Elbow and wrist, eversion	8	Face
14	Deviation of eyes rt	9	Face
15	Convergence of eyes	10	Lids opened
16	Lids twitch, pupils dilate	13	Eyes to lt slight
17	Lids, followed by arm	15	Eyes to lt
18	Shoulder, after long latency	16	Eyes to lt slowly
19	Wrist and elbow	17	Eyes to rt
21	Extension of wrist	18	Eyes to lt lids open, pupils dilate
22	Upper arm and face	19	Lids open, then close
23	Eyes turned to lt	20	Adduction and flexion of forearms
25	Lt eye turned rt	25	Vertical nystagmus, lachrymation
27	Rt shoulder	26	Lids open, eyes move down, homolateral arm moved
28	Upper lip, slight	27	Convergence of eyes, flexion of rt extension of lt arm
30	Extension of wrist, with after-discharge	31	Extension of wrist
31	Wrist extension, followed by elbow	32	Extension of wrist
32	Upper lip, then arm	33	Internal rotation, shoulder
33	Rt eye to lt	34	Pronation, wrist
34	Convergence, pupils dilate spread to lower jaw	37	Abduction, thumb
35	Upper lip	47	Extension of wrist, then thumb
37	Diffuse shoulder	51	Eyes opened, chewing
38	Upper lip, shoulder, elbow	52	Extension wrist, then supination
41	Wrist extension	53	Abduction, shoulder
43	Wrist	59	Abduction, wrist
44	Wrist and elbow	60	Extension and eversion, wrist
46	Pronation	61	Shoulder and elbow
47	Flexion of index finger, then wrist	63	Face and neck
		64	Thumb adducted
		66	Eversion and flexion wrist, some eye movements

*Summary* From the intact area 8 characteristic eye movements were elicited From stimulation the depth of the rostral portion of the scar, diffuse slight movements which were of the type elicited from area 6 of an intact hemisphere, appeared Movements of hands, wrists and forearms were obtained from the posterior lip of the central sulcus in areas 3 and 1 Strychnine placed in an excitable point within the scar did not increase excitability This was taken to indicate that excitability at this point was due to spread on scar tissue and not to active cells within this area

ablation of left areas 4 and 6 Sept 30, 1939, ablation of right areas 4 and 6 Oct 30, 1939, cord transection at Th 5 Jan 2, 1942

Following the cortical ablations during the 6th and 7th months of life this animal recovered sufficient motor skill so that it was able to feed and carry on ordinary cage activities. It had, however, marked scissors gait in the hind legs and used fingers poorly in voluntary prehension. Resistance to passive manipulation was moderately increased and tendon reflexes were markedly hyperactive. For another purpose the cord had been transected in the mid-thoracic region prior to terminal experiment. Subsequent stimulation on the cortex was consequently valid only in those regions supplying upper extremities or face.

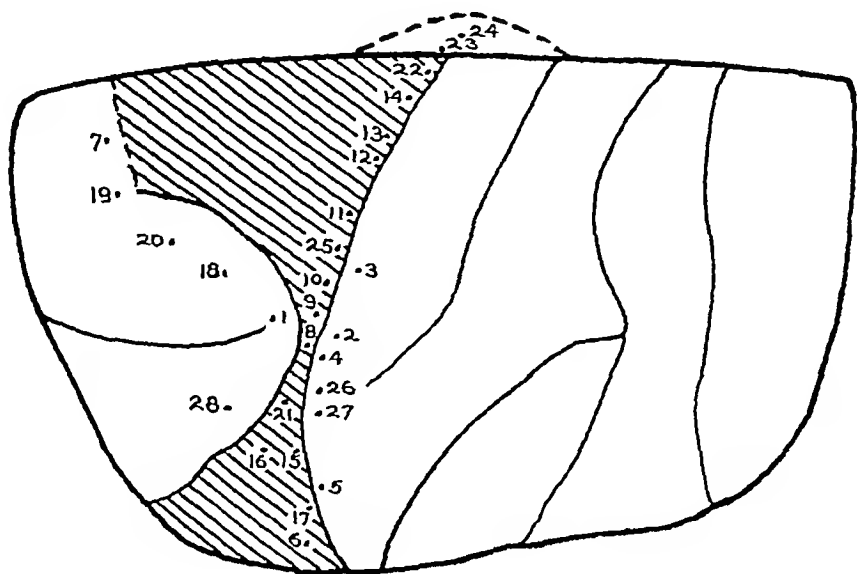


FIG 2

*Stimulation* In Fig 2, the points stimulated on the left cortex are shown. No response was obtained at any point except the following

Point	Response
8	Tongue withdrawn
9	Tongue withdrawn, movement of lower lip
10	Tongue, shoulder and hand movement
12	Tongue, shoulder and arm
13	Tongue withdrawn, fingers flexed
14	Fingers, upper lip
15	Tongue
16	Protrusion of tongue
19	Eyes turned rt
21	Tongue, torsion
22	Upper lip
25	Tongue

*Summary* Normal characteristic movements of lids and eyes resulted from stimulation of area 8. Discrete rapid movements of the lip were obtained with a stimulus of the type usually effective in area 4, from points 14 and 22. Slow generalized movements of the fingers appeared from stimulation of points 12 and 13. They were usually accompanied by tongue and lip movements. There were no discrete finger movements but instead slow diffuse flexion of fingers and wrist, with occasional spread to forearm. Movements of the tongue and lip were obtained from points 8, 9, and 10 easily and quickly and with low volt-

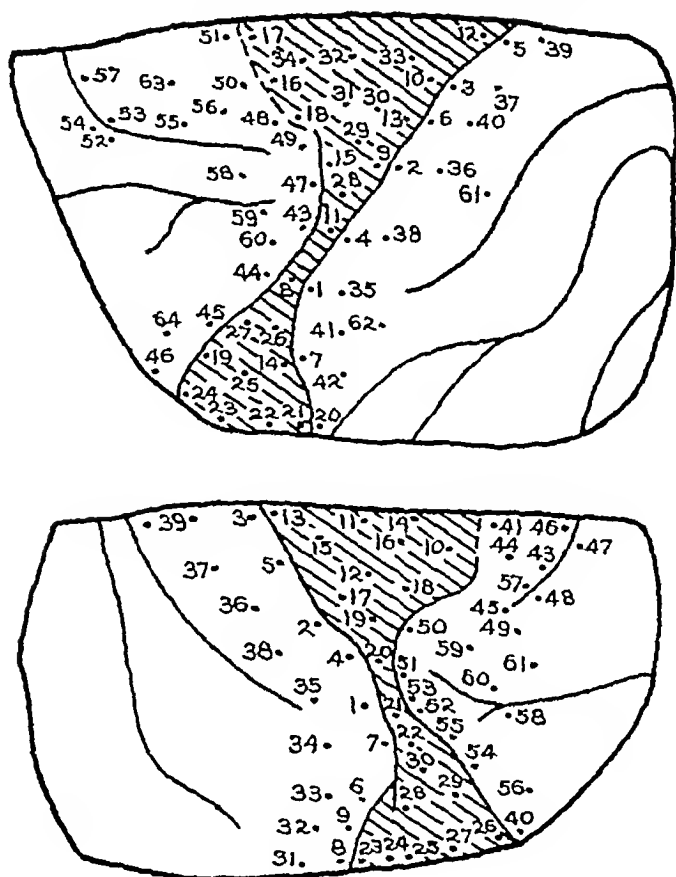


FIG 4

- |   |                                |
|---|--------------------------------|
| 13 Rt upper lip                         | 18 Inward rotation, shoulder   |
| 18 Rt upper lip                         | 19 Inward rotation, shoulder   |
| 28 Supination of wrist                  | 20 Inward rotation, shoulder   |
| 29 Adduction at shoulder                | 41 Eyes lt                     |
| 30 Extension of wrist, finger, then lip | 42 Eyes lt                     |
| 31 Lip followed by hand                 | 43 Eyes lt and up, pupils dil  |
| 32 Lip                                  | 44 Eyes down, rt pupils dil    |
| 33 Extension of wrist                   | 45 Convergence                 |
| 34 Lip, slight                          | 46 Lids blink                  |
| 43 Eyes to rt                           | 50 Convergence of eyes, slight |
| 44 Eyes to rt                           | 56 Eyes to lt                  |
| 51 Dil of pupils                        | 57 Eyes to rt                  |
| 58 Eyes moved to center                 | 59 Eyes lt pupils dil          |
| 63 Eyes rt and up, lachrymation         | 60 Eyes to lt                  |

*Summary* Area 8 was intact and gave characteristic responses. Within the scar, movement of wrist and hand were obtained from one area. These were diffuse and irregular. Areas 3 and 1 on the posterior lip of the central sulcus were excitable. All movements were in the arms and face and were diffuse and generalized. In this animal as in the preceding three, the voltage threshold of stimulus producing movement was high, far higher than in

*Experiment 4 (I 60) July 18, 1942 Male, 2 years, weight 2.3 kg Born Apr 17, 1940  
Ablation of left areas 4 and 6, May 6, 1940, of right areas 4 and 6 Sept 20, 1940*

The motor deficit of this animal during the year before the terminal experiment was stationary and of the same order as that of the animal in Expt 3 but not as extreme. This last was because the ablations were serial and not simultaneous. There was moder-

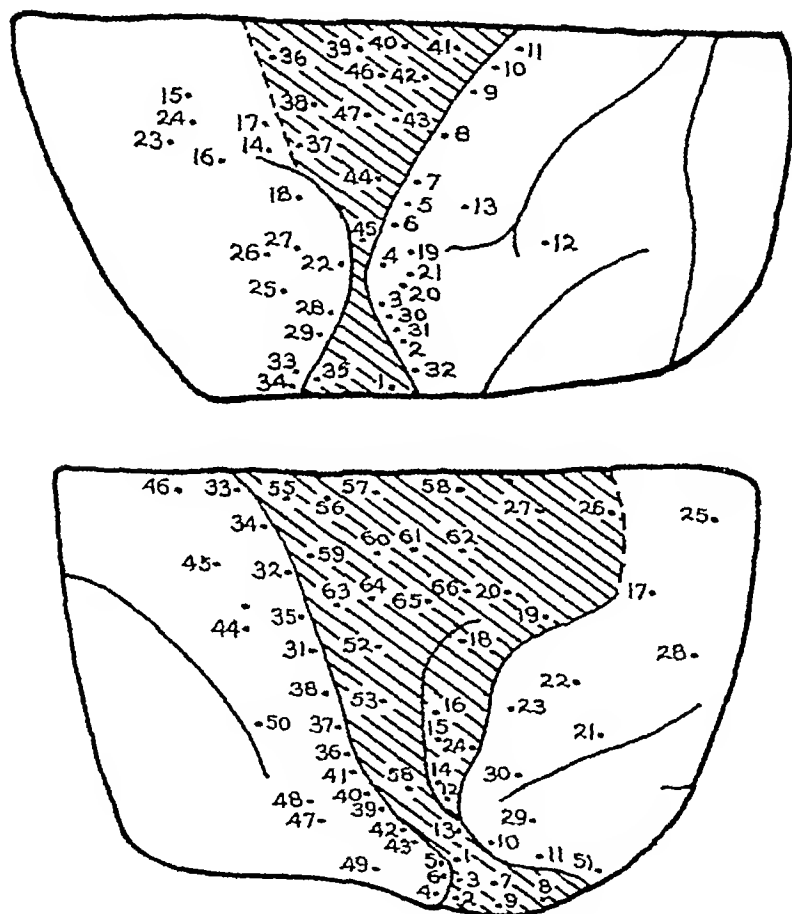


FIG 3

ate spasticity and marked crossing of the hind legs in walking. The forelimbs were everted, the hands and fingers flared widely. Fingers were used awkwardly for voluntary prehension.

*Stimulation.* Figure 4 shows the points stimulated in both left and right sides. Those from which responses were obtained were as follows:

Lt Cortex		Rt Cortex	
Point	Response	Point	Response
2	Face	10	Hip and shoulder
3	Face	11	Leg, tail and shoulder
8	Face	12	Extension of forearm
9	Face, then wrist and extension, finger	15	Extension, wrist and fingers
10	Extension, fingers	16	Flexion, elbow, then hand
11	Thumb extension then wrist	17	Evulsion forearm



tissue and furthermore, the same property appeared in all other excitable areas with the exception of area 8, and, in one instance (Expt 5), area 6. The spread of excitability did not confirm in time relations or in geographical distribution to that which would be expected from facilitation. It appeared directly related to the diffuseness of response to all stimuli. It may be related also to the lack of discreteness in movements during life. (iv) In every instance the excitable regions were those bordering on the scar and those which, in the intact cortex also, are excitable.

A great number of the movements were of tongue, lip and face and were directly related to the more complex movements produced by stimulation of area 6b. Others appeared on stimulation of the post-central gyrus, but were more diffuse and required higher threshold for response than were elicited from the same region of an intact cortex. In the three animals in which the lower extremities were responsive this obtained in the leg area as well as in arm and face. Movements characteristic of stimulation of area 6 were produced from the animal in which area 6 had not been removed at operation (Expt 5) and did not differ in any way from those produced from the same area of an intact cortex. In the other animals occasional diffuse arm, neck and shoulder movements occurred associated with movements of the eyes. These likewise can be produced at the junction of areas 6 and 8 in the intact cortex.

*Causes of differences* Stimulation of the control cortex and those of the four animals operated in infancy demonstrated marked differences between the two types since much more movement was produced from tissue around the scar in the latter than in the control. This would be attributed to any one of several causes.

(1) The most obvious possibility is that the extirpations of the four animals operated on in infancy were less complete than that of the control. This is unlikely for the following reasons: (a), grossly, at autopsy, the scar and the surrounding tissues were the same in extent on all five animals, (b), examination of the histological sections of all five preparations showed about the same extent of each lesion. In every instance there were large motor, possibly Betz cells, on the posterior lip of the central sulcus, on the inferior lip of the cingulate sulcus and, in a few instances, in the anterior lip of the arcuate sulcus. They were not different in size nor in number from the cells of the same type normally found in these sites in the macaque cortex. There was no other indication of any histological difference between the cortex of the animal operated on late in life and those operated on in infancy, (c), previous findings on a large number of monkeys operated on either in infancy or later have been the same, namely that there is always a great deal better motor function in the animals operated on in infancy than in the adults, and that this is without any demonstrable difference in anatomy or histology.

(2) The second possibility is that there is a change in the excitability of the remaining tissue normally concerned with motor function. From the

the normal cortex, both in the scar tissue and the surrounding regions with the exception of area 8. This latter area normally requires a stimulus of longer pulse form and longer duration than do areas 6 or 4. There was no detectable alteration of the excitability of area 8 in the present instances.

*Experiment 5 (I 24) Mar 16, 1942 Male, weight 3.4 kg Born Apr 24, 1938 Ablation of left areas 4, 3, 1 and 2, Dec 9, 1938, ablation of right areas 4, 3, 1 and 2, cord transection at Th 5, Feb 25, 1942*

After the bilateral cortical ablations and preceding the cord transection (which was done for another purpose) this animal had definitely deficient motor performance, although it was not nearly as disabled as those of the preceding three experiments. It was able to use its hands accurately for feeding, although fine movements of prehension were impossible. It ran and jumped awkwardly. Placing and hopping reactions were absent on both sides. It was moderately spastic. To save space the map and list of points are omitted here and the findings summarized below.

*Summary* On both hemispheres, responses to stimulation of area 8 were normal, as they had been in the preceding experiments. Caudal to the scar from areas 5 and 7, no response to stimulation was obtained in either hemisphere. Movements of face and tongue were elicited from each side lateral to the scar of area 4. There was evident spread to this region from stimulation of the scar. Stimulation of area 6 produced movements of arms and fingers characteristic of those usually produced from an intact hemisphere. No suppression was produced in what might have been area 4-s. It was therefore assumed that this area had actually been destroyed at operation.

## DISCUSSION

The result of cortical stimulation in the above five instances is sufficiently consistent to be considered valid and therefore merit discussion.

*Stimulation of adult animal (control)* Stimulation of the cortical tissue about the scar in the older animal produced a characteristic and normal response of eyes and lids from the intact area 8, and of the arm from what was possibly a remnant of the rostral part of area 6, but nothing from either the scar tissue or the postcentral gyrus. These findings confirm many chance observations made previously during stimulations of a large number of cortices of adult or nearly adult monkeys after cortical ablations made, usually, just prior to the stimulation, with one exception, namely, that the postcentral gyrus is sometimes but not always excitable. In an intact cortex of *Macaca mulatta* under proper dial anesthesia as were these animals, stimulation of the posterior lip of the central sulcus will usually produce movement as will stimulation much farther caudally in the parietal lobe.

*Stimulation of cerebral cortices of animals operated in infancy* Stimulation of these cortices produced much more movement than did that of the older animal. This might be expected and is consistent with the much greater adequacy of motor function of the animals operated on at early age. Movements elicited from all four "infants" had the same characteristics. (i) In all five animals the excitability characteristics of area 8 were alike and were the properties normally found on stimulation of area 8 in an intact cortex. (ii) Stimulation elsewhere always required a stimulus of much greater intensity and often of either longer duration or of longer pulse form than normal. (iii) Responses were always diffuse and poorly localized. There was obvious conduction of stimulus along scar tissue such that a given response (i.e. tongue, Expt 2) could be produced from a wide area of scar tissue. This spread of stimulus seemed greater than that which is found in any scar



present data this seems to be the most probable, for, in the present four cases the tissue surrounding areas 4 and 6, or the scar left by their ablation, was more reactive to electrical stimulation than is that of the intact cortex. However, no excitable region or regions were found in these cortices which would not be excitable in the intact cortex also. The difference was one only of degree. The most marked change in those animals operated on in infancy was the great spread of response from stimulation of one point, and, along with this, the similarity of the response obtained from a number of adjacent or fairly widely separated points.

The above explanation of these phenomena fits both the excitability characteristics of the cortices, and the functional characteristics of motor performance in these animals. It is one that has been offered before and has been described previously in the literature, namely that there is a reorganization of remaining tissue within a partially damaged functional unit (1), that of the motor system in this case. There is, at present, no evidence for anatomical reorganization accompanying this although, in the presence of functional reorganization, some anatomical change permitting wider spread of impulses through synaptic connections is still possible.

#### SUMMARY

1 Stimulation of the cerebral cortices of *Macaca mulatta* from which areas 4 and 6 had previously been removed has revealed marked differences in excitability of the cortex from which the motor areas have been removed in infancy as compared to that from which motor areas have been removed later in life.

2 The cortex of the animal with motor areas removed in infancy has greater excitability in the regions surrounding areas 4 and 6, namely the posterior lip of the central sulcus, areas 6b and 6a than has either the normal macaque cortex or that of the animal from which the motor areas have been removed at a later age.

3 Movements elicited from these regions are more diffuse and require a higher threshold stimulus than do these regions in the intact hemisphere.

4 No regions other than those known to be excitable in the normal animal were found to be excitable in these preparations.

5 The changes in excitability in the animals operated on in infancy are consistent with the well developed motor performance of such animals during life.

6 They are consistent with functional reorganization within a partially destroyed motor system. There was no evidence of anatomical reorganization.

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kept warm with a hot bottle, active galvanic responses can be obtained in this way for many hours

The chain was stimulated with a current from an inductorium (Harvard Apparatus Company) and a 1.5 volt dry cell

## RESULTS

*Response to single shocks* Single induced shocks applied to the sympathetic chain near L2 and L3 invariably caused the central pad of the hind foot to give off only single monophasic galvanic responses. In 10 cats the response had an average latent period of 0.6 and a duration of 5.0 sec. The amplitude varied with the intensity of the current as will be shown. Figure 1 shows that the rise of the response was sharp while the fall was relatively gradual.

*Response to make and break shocks of varying intensity* Figure 1 gives a typical record. The vertical lines spaced 1 mm apart show time in  $\frac{1}{5}$  seconds,

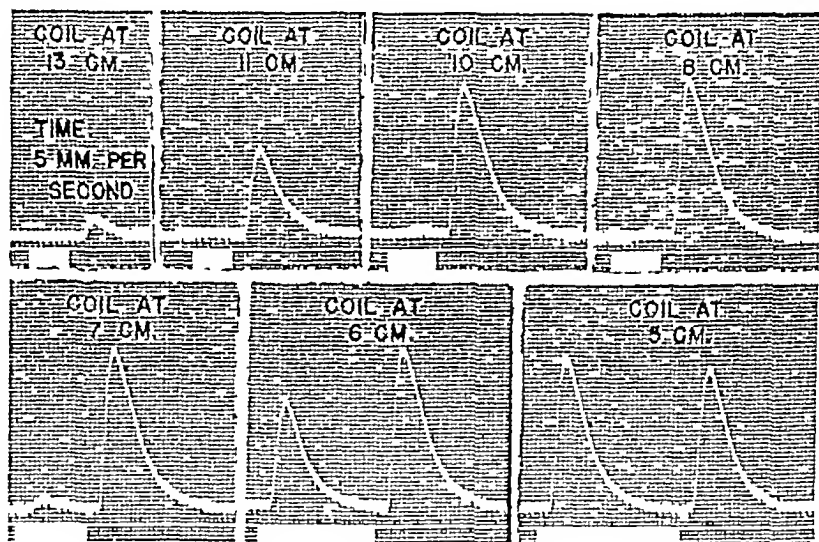


FIG 1

that is, 5 spaces are equal to 1 sec. The white blocks at the bottom of the record show the make and break of the stimulating circuit. For the first record the secondary coil was set at 13 cm, that is, the weakest current that can be obtained on the Harvard inductorium unless the secondary coil is turned on its axis. The 'make' had no effect, the 'break' produced a small current. With the coil at 11 cm the 'make' still failed to give any response, while the amplitude of the 'break' response was considerably increased. With the coil at 10 cm the 'make' again failed to give response, while the 'break' gave a maximum response. At 7 cm the 'make' gave a small response, and at 6 cm a fairly large response. At 5 cm the 'make' response was larger than the 'break'. The latent period did not change with the intensity of the cur-

# SWEAT GLAND RESPONSES TO SYMPATHETIC STIMULATION STUDIED BY THE GALVANIC SKIN REFLEX METHOD\*

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OUR KNOWLEDGE is still limited regarding the nature, intensity and frequency of the nervous impulses which reach the sweat glands from the sympathetic nervous system. The lack of an adequate method of measuring sweat gland responses may in large part account for this lack of knowledge.

In the following experiments we used the galvanic skin response of cats to measure sweat gland activity produced by electrical stimulation of the sympathetic chain. With this method it proved possible to determine several simple principles regarding the sympathetic nerves and sweat gland relationship.

## METHODS

To keep the cats quiet while the records were being taken we placed them in a frame which was originally built for this purpose by Dr. Sarah Tower. A pillory arrangement at one end firmly secured the head and prevented the animal from biting, while the fore and hind legs were either held by an assistant or were tied with strips of muslin bandage to the upright parts of the frame (1).

The electrodes consisted of  $\frac{1}{4}$ -inch zinc discs covered with a paste made of kaolin and saturated zinc sulphate solution. The paste served to make a good electrical connection between the electrode and the skin. One electrode was fastened by means of a small ear ring clip to the pinna of the ear. The other electrode covered with an even layer of paste was placed over the central pad of one of the hind feet. However, before the electrodes were applied the foot was bandaged with a strip of rubber dam. A small hole in the dam allowed only the large central pad to protrude, thus preventing the paste from coming into contact with the small pads and with the skin between the pads. A second bandage of rubber dam around the foot held the electrode in place over the central pad.

The electrodes were connected to a portable string galvanometer (Cambridge Instrument Company) with which photographic records of the galvanic response from the central pad of the foot were obtained. Since no external current was used, the galvanic response consisted of a difference in potential between the electrodes.

For unknown reasons some cats show little or no galvanic activity even in response to direct stimulation of the sympathetic chain. We found that by taking records of the spontaneous galvanic waves before exposing the sympathetic chain such unresponsive cats could be eliminated. Cats which show active galvanic waves before going under the ether anesthesia will invariably show active galvanic responses to stimulation of the sympathetic chain.

When the galvanic activity of the cats had been satisfactorily established, they were put under a light ether anesthesia without removing the head from the pillory or the electrodes from the feet. The sympathetic chain was exposed retroperitoneally through an incision along the line of the ileum and over the thigh of the hind leg. When the chain between the 2nd and 3rd lumbar ganglia had been freed from the surrounding tissue, it was placed in an insulated bipolar shielded electrode (Harvard Apparatus Company) and the incision was closed in such a way as to avoid any pull on the chain. When the cats are

\* This work was carried on under a grant from the John and Mary R. Markle Foundation.

plied to the cut surface of the spinal cord and brain stem also produced only monophasic currents (2, 3, 4). Biphasic or multiphasic currents were obtained only from stimulation of the intact cortex.

In human beings monophasic currents are obtained from a painful stimulation such as a pin prick, shot, etc. These results would indicate that to the intact human being these stimuli cause only a single discharge to pass down the sympathetic chain to the sweat glands.

With a more prolonged stimulus, such as is produced by emotional excitement, the galvanic response in human beings tends to have more the shape of the curves which show complete tetanization. From this we may conclude that the rate of nervous discharge to the chain under these conditions must be greater than 2-4 per sec. It could not be as low as 6 per sec., but might be much higher.

Special mention should be made of the great sensitivity of the galvanic response. In some cats a definite response was obtained when the secondary coil of the inductorium was placed not only at the farthest end but also was turned on its axis until it was almost in the vertical position. Under these circumstances the current must have been extremely minute.

Evidence at hand indicates that the galvanic current produced by the stimulation of the sympathetic chain corresponds to a change in permeability of the sweat glands as they become activated, and not necessarily to the amount of moisture present in the sweat gland ducts and on the surface of the skin. If this response depended on the moisture they would have a much longer duration.

### SUMMARY

1. Single induced shock applied to the sympathetic chain (L2 and L3) of cats caused a galvanic current to be given off by the large central pad of the hind foot. This response which was monophasic had an average latent period of 0.6 and a duration of 5 seconds.

2. The threshold for 'make' currents was much higher than that for 'break' currents. With larger currents the amplitude of the 'make' response finally surpassed that for the 'break'.

3. Induced shocks at a rate of 136 to 375 per minute or 2 to 6 per second sufficed to obtain complete tetanization of the sweat glands, as indicated by the galvanic responses.

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rent, but it was the same for 'make' or 'break' shocks. The duration of the response varied somewhat with the value of stimulating current, increasing very slightly with an increase in current. The other 9 cats gave essentially the same type of record.

*Tetanzation of the galvanic response* In these experiments the coil of the inductorium was set at a position which gave an active galvanic response. The inductorium was set for 'make' and 'break' currents. Single combined

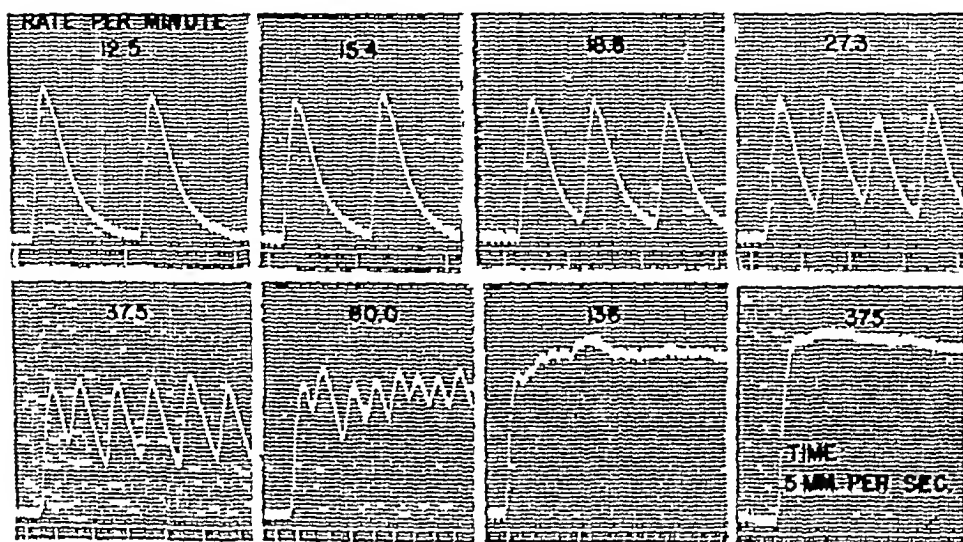


FIG. 2

'make' and 'break' shocks were applied to the *sympathetic chain*. These shocks were produced by the experimenter by a quick touch to the stimulating key.

Figure 2 gives a typical record. When the shocks were applied at a rate of 12.5 per min. the single complete responses were obtained, so also at a rate of 15.4 per min. At 18.8 per min. the response did not quite return to the base line before a second response started. Progressively as the rate of the shocks increased, the responses showed an increased tendency to tetanization. At 375 per min. the responses were completely tetanized. That the response would tetanize at this rate could readily have been predicted from the shape and duration of the single response.

The rate of tetanization, 2-6 per sec., is far below that needed to tetanize striped muscle.

#### DISCUSSION

In these experiments it was found that a single shock applied to the sympathetic chain produced only a monophasic galvanic response. It is of interest that in previous experiments it was found that electric shocks ap-



Horsley-Clarke instrument might have pushed the peri-parotid tissue downward, thus occluding the duct, a modification of the point of fixation was made (Fig 1) This consisted of a round bar holder, which could be adjusted in various directions, held securely on the frame of the instrument, anterior to the usual ear bar holder The tip of the round bar tapered to a small drill head which was fixed to the posterior part of the zygomatic process After this had been done, the right ear bar was withdrawn Thus the new attachment transferred the point of fixation from the right external acoustic meatus to the right zygoma without changing the coordinates After this modification a parotid flow was easily obtained

The stimulating electrode was made of two thin nichrome wires, completely insulated except at the tips, which were about 0.2 mm apart It was introduced into the brain stem

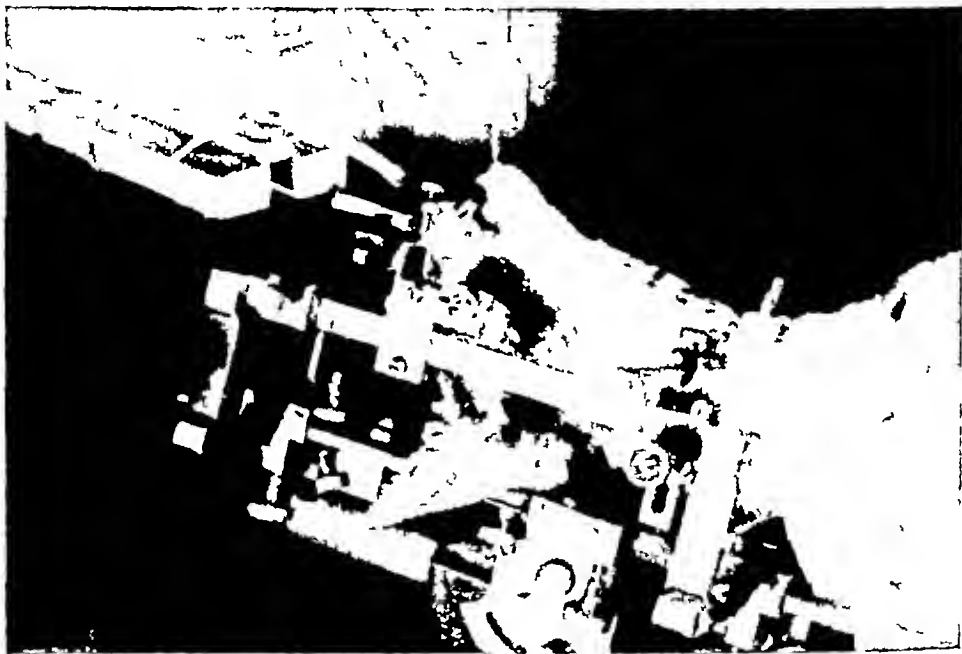


FIG 1 Photograph to show the arrangement of the experiment with the modified attachment of the Horsley-Clarke stereotaxic instrument to the right zygomatic process of a cat The right ear bar has been withdrawn

at a forward inclination of 11 degrees The stimulating current was provided by a Harvard inductorium having a dry cell (1.5 V) and a small rheostat (0.5  $\Omega$ ) in the primary circuit, with the secondary coil set at 13.5 cm For each 15-second stimulus the coordinates and the amount of secretion from both glands were recorded At the end of the experiment the brain was perfused with 10 per cent formalin and serial sections stained by the Weil method The region that yielded the submaxillary or parotid secretion was determined by microscopic examination This is indicated by circles or triangles respectively in six composite diagrams (Fig 2)

It must be noted that although the submaxillary and parotid ducts were cannulated only on the right side, both sides of the brain stem were explored Hence, any crossing of fibers in the brain stem is revealed In the accompanying diagrams submaxillary points in the right half of the medulla are represented on the right side Parotid points are transposed to the side opposite their actual location in the medulla In this way the overlapping of symbols is minimal

# LOCALIZATION OF THE SALIVATORY CENTER IN THE MEDULLA OF THE CAT\*

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## INTRODUCTION

THE EXISTENCE of a salivatory center in the lower brain stem has never been disputed. However, investigators have not been able to agree on its precise anatomical location and its axonic course. In 1902, Kohnstamm (6), using the method of retrograde degeneration, described in dogs a submaxillary nucleus, with its large cells of the anterior horn type widely distributed in the reticular formation at the caudal level of the facial nucleus. Later, Kohnstamm (7) found a parotid nucleus between the inferior olive and the nucleus ambiguus. The efferent fibers were largely crossed. In 1909, Yagita and Hayama (17) repeated these observations and found that after sectioning the chorda tympani a group of cells of the visceral motor type underwent chromatolysis. These cells were located in the lateral reticular formation, at the level of the facial nucleus, ventral to the lateral vestibular nucleus of Deiters and medial to the spinal trigeminal nucleus. Yagita (16) described a parotid nucleus forming the caudal prolongation of his submaxillary nucleus. Both groups of degenerate cells were homolateral to the cut nerves. Salivary secretion has also been repeatedly noted by a number of investigators on stimulation of the medulla (see review by Langley, 9), but the available data give little help in further identification of the center. It was thought, therefore, that a careful examination of the lower brain stem with the aid of the Horsley-Clarke stereotaxic instrument might yield valuable information as to the location of the center.

While this investigation was underway, Chatfield (2) and recently Magoun and Beaton (10) reported similar studies in cats and monkeys respectively. The results of our study are in general agreement with their reports, but certain differences will be noted.

## METHODS

Thirty-five cats were included in this series. The animals were anesthetized with 35-40 mg of nembutal per kg of body weight, intraperitoneally. After the cerebellum was exposed, the Horsley-Clarke instrument was fastened to the cat's head. The submaxillary and parotid ducts on the right side were cannulated with fine glass cannulas, and each duct was connected to a horizontally-placed, graduated capillary tube. The secretion was recorded in cm and the rate of flow was later converted into cc per min. The right cervical sympathetic nerve was cut in order to isolate the parasympathetic effects.

In the early experiments, no parotid secretion was obtained either on stimulation of the medulla or by injection of pilocarpine. Since it was believed that the ear bar of the

\* A preliminary report of this work was presented before the American Physiological Society, Boston Fed Proc Amer Soc exp Biol, 1942, 1, 88-89.

currents induced a copious flow of saliva. The swallowing or respiratory movements which occasionally accompanied salivary secretion were not causally related to the salivary flow. This was shown by experiments in which stimulation of an appropriate region yielded salivation associated with deglutition or respiratory movements, while stimulation of the corresponding contralateral region yielded similar extraneous responses without comparable salivary secretion.

After a 15-second period of stimulation at a favorable location, as much as 1.2 cc per min of secretion was collected from the submaxillary gland, and as much as 1.1 cc per min from the parotid. The locations which, upon stimulation, yielded a secretion of more than 0.25 cc per min are represented in the diagrams by large solid circles (submaxillary) and large solid triangles (parotid), representing an average of 0.5 cc per min and 0.4 cc per min respectively.

A typical submaxillary flow would commence about 2 seconds following stimulation, that from the parotid usually had a longer latency. The flow for the first 15-second period was usually most rapid, decreasing for each 15-second period thereafter. Consequently the flow per min as calculated from the first 15-second period is greater than the amount of saliva collected if the stimulus actually lasted for one minute. The after-discharge was sustained for about 3-4 seconds, and rarely longer. For the less favorable regions (0.1 cc to 0.25 cc per min), the latency was slightly longer and in many instances the flow slowed down considerably before the end of the 15-second stimulus.

A striking distribution of the responsive locations of the submaxillary points in two groups was revealed at the bulbar level. This is shown in Fig. 2a, in which a medial group lies in the vicinity of the facial genu, and a lateral group in the area corresponding to the spinal trigeminal nucleus and tract. The intervening region was surprisingly silent when stimulated. At a level about one mm caudad (Fig. 2b), the two responsive regions adjoined. The area medial and slightly dorsal to the spinal trigeminal nucleus and its caudal extension form a very excitable region, which, on stimulation, produced an abundant submaxillary secretion. This area corresponds to the dorso-lateral portion of the lateral reticular formation and is believed by Papez (12, p. 222) to constitute the rostral component of the salivary nucleus. In the section through the facial nucleus and superior olive and at the level of *nervus intermedius* (Fig. 2c) the medial region was less responsive than the corresponding rostral area. At this level (Fig. 2c) the responsive points were most numerous in the dorsolateral portion of the lateral reticular formation, although scattered points were present in the spinal trigeminal area. At more caudal levels (Fig. 2e and 2f) salivary secretion was occasionally obtained when the area of the solitary fasciculus and its nucleus was stimulated.

The configuration of the parotid points is very similar to that of the submaxillary, except that the corresponding parotid regions appear roughly one or two mm more caudally. Profuse secretion was not obtained following

## RESULTS

The most rostral level included in the exploration was at the anterior border of the pons. Stimulation of the brain stem at the pontile level even with strong currents produced no appreciable flow of saliva from either the submaxillary gland or the parotid gland, whereas stimulation of the responsive regions at the successive levels in the medulla even with weak

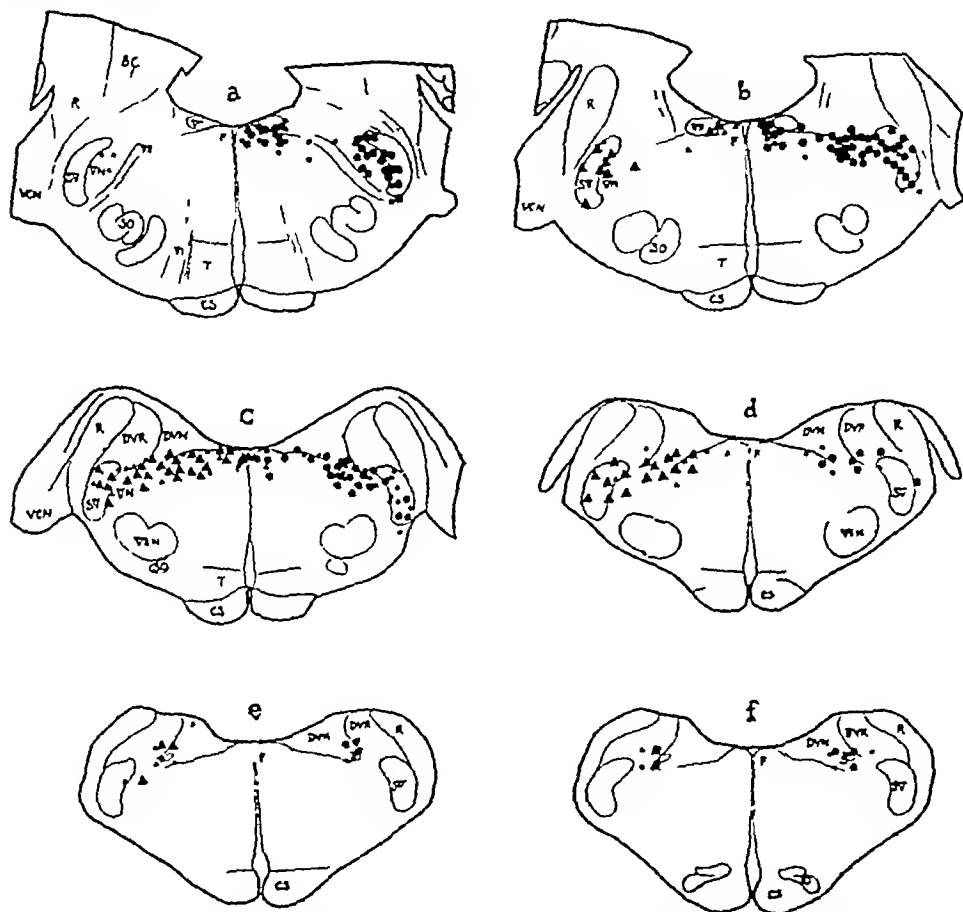


FIG 2 Diagrammatic representation of results obtained upon stimulation of the medulla of 35 cats. The sections are about one mm apart. Each large solid circle or triangle represents a response of salivary flow at a rate of more than 0.25 cc per min, and each small solid circle or triangle, at a rate of 0.1 cc to 0.25 cc per min. Secretion of less than 0.1 cc per min is not represented. Circles indicate responses from the right submaxillary gland and triangles, responses from the right parotid gland but transposed to the opposite side. Abbreviations are: BC—brachium conjunctivum, CS—cortico-spinal tract, DVN—dorsal or medial vestibular nucleus, DVR—descending vestibular root, F—medial longitudinal fasciculus, IO—inferior olive, R—restiform body, S—Solitary fasciculus, SO—superior olive, SV—Spinal tract of the trigeminal nerve, Tr—trapezoid body, VCN—ventral cochlear nucleus, VM—spinal nucleus of the trigeminal nerve, VI—abducens nerve, VII—facial nerve, VIIIN—facial nucleus.

reasonable to attribute the salivatory effect obtained on stimulation of the medulla in these experiments to the excitation of the parasympathetic secretory components, including the center itself

According to Miller (11) the threshold for eliciting salivary secretion by stimulation of the floor of the fourth ventricle or by reflex stimulation of chorda-lingual or glossopharyngeal nerves is low, whereas the threshold for salivary secretion induced by stimulation of the gastric vagus or sciatic nerves is high. These observations suggest that stimulation of the intramedullary courses of the afferent "taste" fibers should yield salivary secretion\*. In our experiments stimulation of the region corresponding to the solitary fasciculus and its nucleus was found occasionally to yield abundant secretion from both the submaxillary and the parotid glands. The salivary secretion resulting from stimulation of the spinal trigeminal area appears to be more complicated in that, while stimulation of the rostral portion of the trigeminal area produced abundant secretion, stimulation of the more caudal portion induced no salivary secretion. The presence of a number of rootlets of the nervus intermedius and the glossopharyngeal nerve which pass only through the rostral spinal trigeminal area suggests that these fibers might account for the responses obtained on stimulation. However, on careful examination, it appears doubtful that a copious salivary secretion would follow a punctate stimulation of a few of the widely distributed efferent rootlets. Furthermore, the isolated groups of positive points from which salivary secretion was obtained in the most rostral regions of the spinal trigeminal area (Fig. 2a) would not have been found if the salivary secretion had resulted from the excitation of efferent fibers passing through the stimulated area.

Various investigators have suggested that different branches of the trigeminal nerve terminate at different levels of the spinal trigeminal tract. For example, it is believed that the mandibular branch occupies only the rostral portion of the spinal trigeminal tract and does not extend down to the caudal region (3). This conception would explain the refractoriness of the caudal portion of the spinal trigeminal area (Fig. 2e and 2f) when it is stimulated with weak currents. The evidence indicates, therefore, that stimulation of the spinal trigeminal tract and nucleus at a specific level (presumably mandibular) produces salivary secretion. Nevertheless, the response obtained on stimulating this area may be the result of excitation of efferent rootlets as well as of the secondary afferent fibers.

The areas dorso-medial (referred to below as medial) and dorsal (referred to below as lateral) to the facial nucleus constitute regions in which the salivatory nuclei may be located. In favor of the medial position is the fact

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\* Our experiment differs from that of Miller in that we used nembutal anesthesia instead of decerebration. It is known that barbiturates decrease the salivary secretion (14), but this action is thought to be exerted on the secretory cells. Percy and Weaver (13) found that barbital in quantities sufficient for surgical anesthesia causes little or no depression of other visceral reflexes.

weak stimulation at the level of the outgoing limb of the facial nerve (Fig. 2a). More caudally, at the level of the facial nucleus and the superior olive (Fig. 2c) parotid secretion was more easily obtained. It was noted that stimulation of the same locus often produced secretion from both submaxillary and parotid glands. In one experiment a definite but small parotid secretion was obtained when the contralateral medial floor of the fourth ventricle was stimulated (Fig. 2c). At the next level through the middle portion of the facial nucleus and through the glossopharyngeal nerve (Fig. 2d) the responsive region corresponds to the dorsolateral portion of the lateral reticular formation and to the spinal trigeminal nucleus and tract. At the lower levels (Fig. 2e and 2f) stimulation of the area of the solitary fasciculus occasionally was found to yield good secretion. It is interesting to note that stimulation of the area between the inferior olive and the nucleus ambiguus designated by Kohnstamm as the inferior salivatory nucleus (8) did not cause any definite flow of saliva from the parotid gland.

Responses from both the submaxillary and the parotid glands were obtained when portions of the homolateral medulla were stimulated. Scanty secretion from either gland, however, was obtained when the contralateral side was stimulated. Even when strong currents were used, no flow greater than 0.25 cc per min was observed. Under such conditions the area of homolateral reactivity was considerably scattered, and vigorous respiratory changes and contractions of various muscles occurred.

The mechanism of the salivary secretion obtained following central stimulation has also been investigated. For instance, the intravenous injection of 0.1 mg of atropine promptly and completely stopped the salivary secretion produced by medullary stimulation. In a number of experiments the chorda tympani was sectioned before it joins the lingual nerve. No submaxillary secretion could be elicited by stimulation of the medulla at a point which previously yielded a copious flow of saliva.

## DISCUSSION

It has long been known that the salivary glands receive their innervation from two general sources: the sympathetic and the parasympathetic. Stimulation of either nerve supply produces secretion. In this series of experiments the cervical sympathetic fibers have been severed. Consequently the responses obtained cannot be due to stimulation of the medullary sympathetic or descending hypothalamic pathways. It is also well established that the vasodilation following the stimulation of the chorda tympani has no causal connection with the secretion of saliva by the gland in consequence of the stimulation (1). The fact that in our experiments a small dose of atropine abolishes the secretion produced by stimulation of responsive points in the medulla also indicates that vaso-dilation, which remains active after atropine,\* is not the primary factor causing secretion. Hence, it is

\* Henderson and Loewi (4) found that atropine in small doses paralyzed only the secretory but not the vaso-dilator fibers of the chorda tympani.

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that other general visceral efferent neurones, such as the Edinger-Westphal nucleus of the third cranial nerve and the dorsal motor nucleus of the tenth cranial nerve, are located along the axis. However, if this position is accepted, it becomes difficult to explain the abundant submaxillary secretion which results from stimulation of wide areas of the lateral reticular formation directly dorsal to the facial nucleus (Fig 2c). There is no reason to assume that the efferent and afferent fibers, if scattered over a wide region, would yield, upon stimulation such vigorous responses in a rather circumscribed area. If, on the other hand, the nuclear masses are located in the lateral position, the axons will have to travel, like those from the facial nucleus and the nucleus ambiguus, rostrally and dorso-medially before making their exit. The increased responsiveness in the lateral area may then be readily accounted for by the stimulation of the cells and the efferent fibers after they have made the turn, as well as the secondary afferent fibers from the nucleus of the solitary fasciculus and the spinal trigeminal nucleus. It is recognized that the evidence presented here in favor of the lateral location of the salivatory center is at best presumptive. Nevertheless, this contention is strengthened by Yagita's anatomic studies of the salivatory nuclei in the dog (17) and by Kimmel's recent ontogenic studies of the same nuclei in the rabbit (5).

#### SUMMARY

The lower brain stem of 35 cats has been stimulated with the aid of the Horsley-Clarke stereotaxic instrument. It was found that a copious salivary flow is easily elicited from the homolateral glands when the medulla is stimulated with a weak current.

Analysis of the responsive locations reveals that salivary secretion can be obtained by stimulation of the intramedullary visceral (oral) afferent system, such as the solitary fasciculus and its nucleus and certain portions of the spinal trigeminal nucleus and tract (mandibular division?). On the efferent side, the distribution suggests that the salivatory nuclear masses might be either in the medial position caudal to the facial genu, or more likely, in the dorso-lateral region of the lateral reticular formation, dorso-medial to the spinal trigeminal nucleus, and dorsal to and at the level of the facial nucleus. In the latter case, the efferent fibers must travel dorso-medially before they turn and make their exit in the ventro-lateral portion of the medulla. In any event, there is no sharp division of the centers of the salivatory nerve fibers carried in the seventh and ninth cranial nerves. The rostral portion supplies the submaxillary glands, and the caudal portion the parotid. There exists an intermediate portion, stimulation of which yields both submaxillary and parotid secretion.

The author wishes to thank Dr. Walter S. Root for his invaluable criticism of the manuscript.



additions were made cytochrome c, diphosphopyridine nucleotide,\* cocarboxylase (diphosphothiamin, prepared by Merck) and adenosinetriphosphate, prepared from rabbit muscle according to the method of Lohman (6)

*Pyruvic dehydrogenase* The method of Quastel and Wheatley (11) was used, in which the reduction of ferricyanide by hydrogen atom leads to the formation of acid. In bicarbonate solution this liberates  $\text{CO}_2$  which is estimated manometrically. The method was used as described for succinic dehydrogenase (9). The failure of Quastel and Wheatley to determine pyruvic dehydrogenase may be due to insufficient concentration of some coenzymes necessary for this enzyme system (5, 11). The substances used were those mentioned for the work with pyruvic oxidase.

*Preparation of the tissue* In view of the high sensitivity of some respiratory enzymes special precaution has to be used for the preparation of the material. The trunk was rapidly cut and put into sea water of about  $+2^\circ\text{C}$ . The preparation of the isolated giant axon requires considerable time and some preliminary experiments indicated that during this time the rate of respiration decreased. The isolation of the giant axon was therefore abandoned and the following procedure was adopted. The whole trunk after having been cooled at  $2^\circ\text{C}$  for 5–8 minutes was put on a cool glass plate and the axoplasm extruded. The respiration of the axoplasm could then be compared with that of the whole trunk as well as with that of the remaining tissue consisting of small nerve fibers, connective tissue and sheath.

*Estimation of dry weights* The dry weights used for the calculation of the  $\text{Q}_{\text{O}_2}$  values were as follows. For the head ganglion 200 mg per gm fresh weight, determined by Nachmansohn and B. Meyerhof (8), for the axoplasm 100 mg per gm fresh weight, determined by Bear and Schmitt (2), for the whole trunk 140 mg per gm and for the remaining tissue 148 mg per gm fresh weight. The figure for the whole trunk is based on the assumption that it has about the same dry weight as a lobster nerve trunk, for which Schmitt, Bear and Silver obtained a value of 140 mg per g (13). For the remaining tissue the figure found by Bear and Schmitt for the envelope of the giant fiber was taken. Although it is possible that the actual values vary slightly from these figures, the difference could alter the final result only slightly.

## RESULTS

### A Cytochrome oxidase

The distribution of the succinic dehydrogenase found in the giant fiber suggests that the bulk of the respiratory enzyme system is located in the axoplasm. This is however only indirect evidence. The succinic-fumaric system may not be necessary in all oxidative processes. On the other hand, there is general agreement that the first activation of oxygen of physiological

Table 1 Cytochrome oxidase in nerve tissue of the squid

Tissue ground in 1.0 cc of 0.1 M phosphate buffer, pH = 7.4. 0.2 cc of p-phenylenediamine, final concentration 0.03 M. 0.2 cc. of cytochrome c, 1.0 mg per cc. Time of observation 30 min.  $t = 23^\circ\text{C}$

Exp No	Head Ganglion		Trunk Containing Giant Axon		Axoplasm		Remaining Tissue	
	Mg Tissue	$\text{Q}_{\text{O}_2}$	Mg Tissue	$\text{Q}_{\text{O}_2}$	Mg Tissue	$\text{Q}_{\text{O}_2}$	Mg Tissue	$\text{Q}_{\text{O}_2}$
1	63.0	-9.5	77.0	-0.98	59.0	-2.86	61.0	-0.25
2	63.0	-9.3	143.0	-0.90	43.0	-3.42	54.0	-0.74
3	39.0	-13.0			30.0	-3.40		

\* Kindly provided by Dr. M. E. Krahle, Lilly's Research Laboratories

# LOCALIZATION OF ENZYMES IN NERVES

## II RESPIRATORY ENZYMES

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### INTRODUCTION

THE HIGH concentration of choline esterase (ChE) at the neuronal surface, which may be demonstrated in the giant axon of the squid, and the parallelism found between the concentration of the enzyme and the E M F of the action potential in electric organs suggest that the release of acetylcholine (ACh) is intrinsically connected with the electrical changes which occur during nerve activity (3, 8, 10). Investigations were therefore initiated to study the distribution of other enzymes known to be involved in the metabolism of nerve. Experiments showed that approximately 90 per cent of succinic dehydrogenase and of the succinic oxidizing system is located in the axoplasm, in contrast to the localization of ChE. There was no evidence that a high concentration of these enzymes occurred at or near the neuronal surface (9). The concentration of vitamin B<sub>1</sub> (as diphosphothiamin), on the other hand, is many times higher on the neuronal surface than in the axoplasm.

In the present paper further observations on the distribution of respiratory enzymes in the giant axon of the squid are described.

### METHODS

**Cytochrome oxidase.** The O<sub>2</sub> uptake was measured by the usual manometric method of Barcroft-Warburg. For the determination of cytochrome oxidase, small conic vessels were used, the total volume being only about 5.0 cc. and the K<sub>2</sub>O under conditions of the experiments 0.30–0.35. Tissue was ground in 0.1 M phosphate buffer of pH 7.4 and 1.0 cc. of the suspension was put into the vessels. The homogenizer of Potter and Elvehjem was used to grind it. During the grinding the tube containing the tissue was kept in ice water. Axoplasm was put directly into the buffer without grinding since it dissolves rapidly in isotonic solution. In 0.2 cc. H<sub>2</sub>O p-phenylenediamine dihydrochloride was added to give a final concentration of 0.03 M. Cytochrome c, prepared according to the method of Keilin and Hartree (4) was added in 0.2 cc. of buffer, the final concentration being 1 mg. per cc. Controls were run without tissue.

**Pyruvic oxidase.** The respiration with pyruvate or glucose as substrate was measured in conic vessels with a center well for the KOH. The total volume was about 7 cc., the K<sub>2</sub>O with 1.0–2.0 cc. of fluid present was 0.5–0.6 cc., 0.1 cc. of 8 per cent KOH were put into the center well. The tissue was either minced or ground in 0.1 M phosphate buffer of pH 7.1. The final concentration of sodium pyruvate was 0.05 M. In a few experiments with minced tissue no other substances were added. In all the other experiments the following

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† Fellow of the Panamerican Sanitary Bureau and the Department of Biophysics, School of Medicine, University of Brazil, Rio de Janeiro.

The results obtained with pyruvate as substrate are given in Table 2. The rate of  $O_2$  uptake in minced head ganglion is nearly the same as if p-phenylenediamine is used as substrate, the  $Q_{O_2}$  values being  $-7.0$  to  $-9.0$ . This indicates that the concentrations of all the enzymes and co-enzymes necessary for the intermediate reactions between the first step of pyruvic acid oxidation and the cytochrome-cytochrome oxidase system are sufficiently high so that they are not limiting factors. The oxidation of pyruvic acid in dialyzed extracts of brain requires, according to Banga, Ochoa

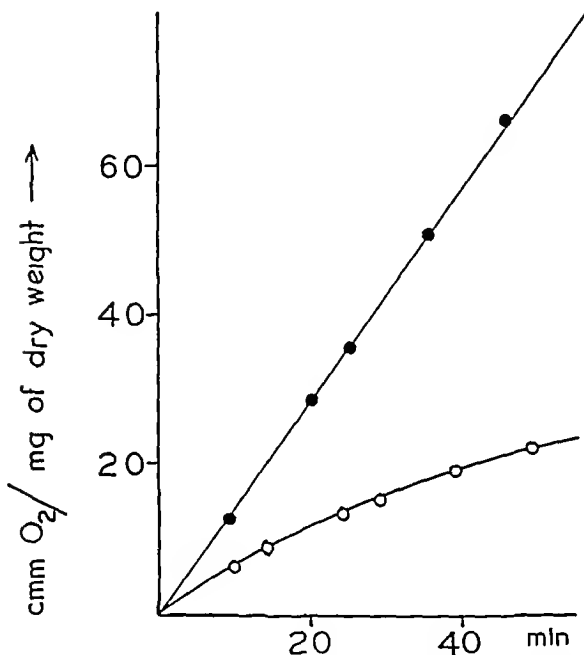


FIG 1 Pyruvate oxidation in the head ganglion of squid. Abscissae: cmm  $O_2$  per mg of tissue dry weight. Ordinates: time in min. ●—● minced, ○—○ homogenized tissue. 0.5 cc of 0.1 M phosphate buffer, pH  $\approx$  7.4. 0.1 cc of sodium pyruvate, final concentration (f.c.) 0.05 M. 0.1 cc of cytochrome c f.c. 1 mg per cc. 0.2 cc of adenosinetriphosphate, f.c. 3 mg per cc. 0.1 cc of diphosphothiamin, f.c. 50  $\mu$ g per cc. 0.1 cc of diphosphopyridine nucleotide, f.c. 10  $\mu$ g per cc.

and Peters (1), at least the following substances: adenylic acid or adenosinepolyphosphate, diphosphothiamin (cocarboxylase), inorganic phosphate in a concentration of 0.05–0.1 molar, a  $C_4$ -dicarboxylic acid such as succinic, fumaric or malic acid in catalytic amounts, magnesium ions and probably, although the evidence is not conclusive, diphosphopyridine nucleotide. But since the oxidation of pyruvic acid in minced ganglion is already as high as the activity of cytochrome oxidase permits, addition of these compounds cannot have any marked effect, and the  $Q_{O_2}$  of Experiment 4 is in fact the same as in the experiments without these additions.

When however the ganglion is ground, the rate of pyruvic acid oxidation falls off rapidly, even in the presence of all the factors known to be of consequence in this process. Figure 1 shows the difference between ground and minced tissue. In this experiment the first reading was made about one hour after the animal was killed. In an experiment with ground ganglion in which the preparation was made with high rate of speed, the first reading was

significance in living cells occurs through the cytochrome-cytochrome oxidase system. It appeared therefore necessary to determine the distribution of cytochrome oxidase.

Cytochrome oxidase has been determined (i) in the head ganglion of the squid as sample of nerve tissue in which cell bodies and synapses are located, (ii) in the whole trunk containing the giant axon, (iii) in the axoplasm extruded from the trunk and (iv) in the tissue of the trunk which remains after the extrusion of the axoplasm.

The results are summarized in Table 1. The head ganglion has a remarkably high rate of oxygen uptake for the tissue of a cold blooded animal. The

Table 2. *Pyruvic oxidase in nerve tissue of the squid*

Minced tissue. In the experiments Nos. 1-3 with the head ganglion and Nos. 1 and 2 with the trunk the tissue was put into 0.8 cc. of 0.1 molar phosphate buffer of pH 7.4. 0.1 cc. of sodium pyruvate was added, its final concentration being 0.05 *M*. In the other experiments the tissue was put into 0.5 cc. phosphate buffer and besides sodium pyruvate the following additions were made: 0.1 cc. of cytochrome *c*, final concentration = 1 mg per cc., 0.12 cc. of adenosinetriphosphate, *f.c.* = 3 mg per cc., 0.1 cc. of diphosphopyridine-nucleotide, *f.c.* = 10  $\mu$ g per cc., 0.1 cc. of diphosphothiamin, *f.c.* = 50  $\mu$ g per cc. In centrifuge well 0.1 cc. of 8 per cent KOH. Time of observation 30 min. *t* = 23-24°C.

Exp. No.	Head Ganglion		Trunk Containing Giant Axon		Axoplasm		Remaining Tissue	
	Mg Tissue	Q <sub>o</sub>	Mg Tissue	Q <sub>o</sub>	Mg Tissue	Q <sub>o</sub>	Mg Tissue	Q <sub>o</sub>
1	42.0	-6.7	47.0	-1.00	57.0	-0.34	73.0	-0.37
2	32.0	-8.9	44.0	-1.22	73.0	-0.92	123.0	-0.88
3	51.0	-8.4	77.0	-1.27				
4	89.0	-9.7	184.0	-1.40				

figures obtained with the whole trunk and with the axoplasm and remaining tissue separately offer evidence that the greatest part of the cytochrome oxidase is localized in the axoplasm. The Q<sub>o</sub> values of the axoplasm (the O<sub>2</sub> uptake per mg. of tissue dry weight) are about three times as high as those of the whole trunk. The difference between the whole trunk and the axoplasm is even more marked than in the case of succinic dehydrogenase. In the remaining tissue the values are lower than in the whole trunk. It has to be borne in mind that while most of the axoplasm is removed from the giant axon, some still remains attached to the sheath. Moreover the small fibers of the trunk retain their axoplasm. Part of the activity of the remaining tissue has therefore to be attributed to the axoplasm remaining in the trunk.

### B. *Pyruvic oxidase*

Since pyruvate, or glucose, increases acetylcholine formation in brain in aerobic conditions, the enzymes necessary for the oxidation of these two substances are of special interest in connection with the problem investigated.

Table 3 *Respiration of the head ganglion of squid*

Substrate glucose, 0.01 molar in 0.1 molar phosphate buffer, pH=7.4 Minced tissue Time of observation 30 min  $t=23^{\circ}\text{C}$

Exp No	1	2	3	4	5
Mg Tissue Fresh Weight	42.0	36.0	42.0	36.0	34.0
$\text{QO}_2$	-8.2	-8.0	-6.3	-7.0	-8.2

sion of the head ganglion. The effect is shown in Fig. 3. In the presence of adenosinetriphosphate, succinic acid and diphosphopyridine nucleotide, the rate of  $\text{CO}_2$  production in the presence of pyruvate is only slightly increased over the rate of control. But if cocarboxylase is added the increase of the

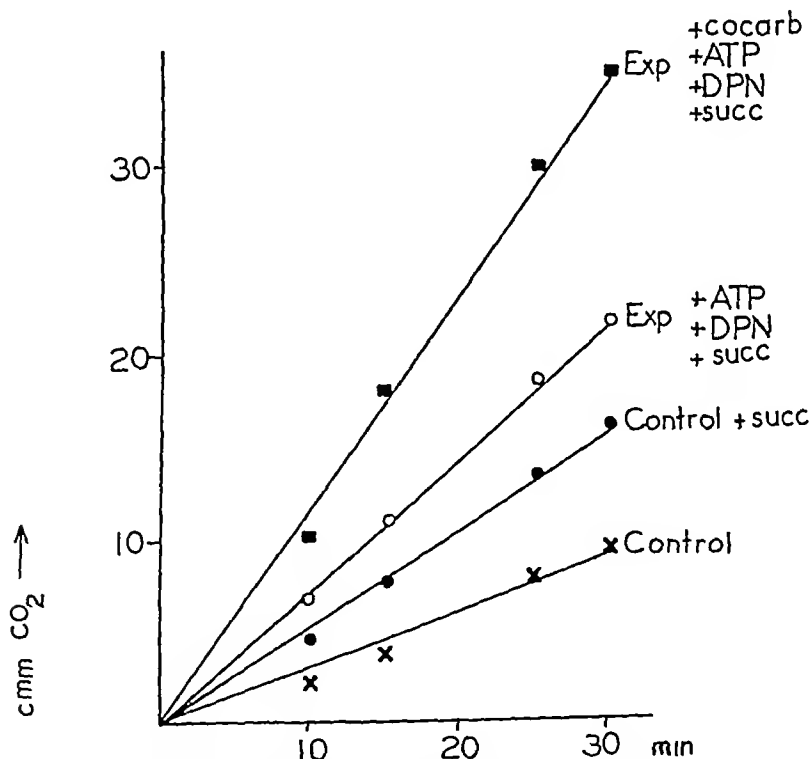


FIG. 3 Effect of diphosphothiamin on pyruvate dehydrogenation with an homogenized suspension of the head ganglion of the squid. In each vessel 1.0 cc of suspension in bicarbonated Ringer. Concentration of substances added: 0.2 of sodium pyruvate, f.c. 0.05 M; 0.1 cc of sodium succinate, f.c. 0.001 M; 0.2 cc of adenosinetriphosphate (ATP), f.c. 3 mg per cc; 0.2 cc of diphosphopyridine nucleotide (DPN), f.c. 10  $\mu\text{g}$  per cc; 0.1 cc of diphosphothiamin (cocarboxylase), f.c. 50  $\mu\text{g}$  per cc; 0.1 cc of potassium ferricyanide in the side bulb.

made 20 min after death, the oxidation rate during the first 30 min was nearly as high as in minced tissue. In the second half hour the rate of the oxidation went down rapidly and did not differ from that of the experiment in Fig 1. Even so the compounds added seem to have some effect as in the brain dispersions of Banga, Ochoa and Peters. In the experiment presented

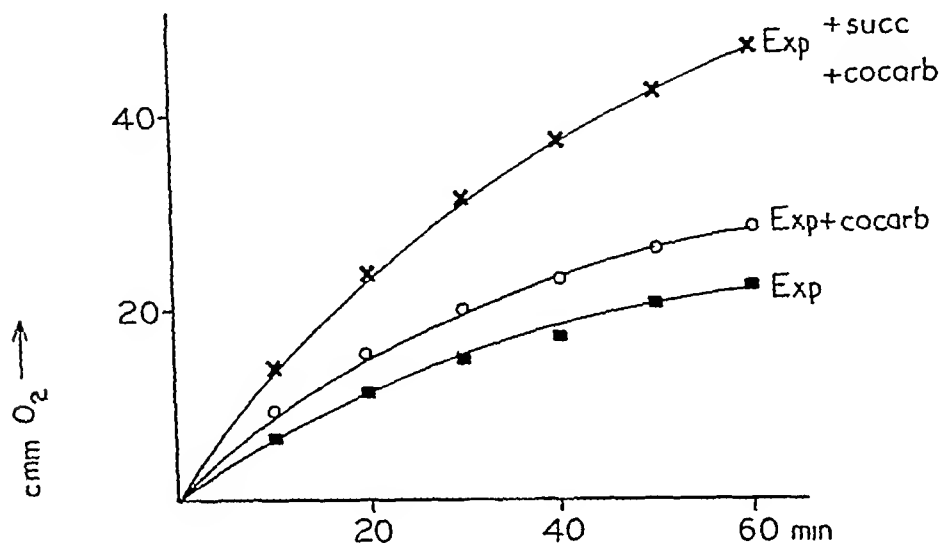


FIG 2 Effect of diphosphothiamin and succinate on pyruvate oxidation with an homogenized suspension of the head ganglion of the squid. The concentration of the substances added was the same as in the experiments shown in Fig 2 and 3

in Fig 2, the rate of  $O_2$  uptake is lowest in the vessel containing pyruvate, adenosinetriphosphate, diphosphopyridine nucleotide and cytochrome c. The rate rises if diphosphothiamin is added and even more if, besides that, a  $C_4$ -dicarboxylic acid (succinic acid) is present.

The difference between pyruvic oxidation in ground and minced tissue indicates that some enzymes necessary for this process are rather fragile and rapidly lose their activity if the cell is destroyed, or there is still an unknown factor which is not present in sufficiently high concentration in homogenized tissue suspension. It can therefore not be expected that pyruvic acid oxidation in the axoplasm is measurable, because the axoplasm dissolves rapidly in isotonic solution. The  $Q_{10}$  values are indeed low.

If glucose is used as substrate with minced ganglion, the rate is the same as with pyruvate (Table 3).

### C Pyruvic dehydrogenase

In brain preparations pyruvic acid can be broken down to some extent by dehydrogenation to carbon dioxide and acetic acid (11). Diphosphothiamin strongly stimulates pyruvic acid dehydrogenation in a homogenized suspen-

the axoplasm as compared with other cells whereas the rate of acetylcholine metabolism is extremely high in the sheath

The oxidation of pyruvic acid, necessary for acetylcholine formation in brain tissue, occurs in the head ganglion and the trunk containing the giant axon at approximately the same rate as the  $O_2$  uptake with p-phenylenediamine. This indicates that the intermediate steps of pyruvic acid oxidation occur at a rate limited only by that of the  $O_2$ -uptake through the cytochrome-cytochrome oxidase system. Direct evidence, however, could not be brought since the oxidation of pyruvic acid falls off rapidly if the cell structure is not intact.

The increase of the rate of pyruvic acid dehydrogenation in presence of diphosphothiamin is of special interest. According to Long about 20 per cent of the pyruvic acid removed by oxidation in brain, can be accounted for as acetic acid, whereas the greater part is completely oxidized to carbon dioxide and  $H_2O$ . In view of the high concentration of diphosphothiamin at the neuronal surface it is possible that a higher percentage of pyruvate is there transformed into acetic acid by dehydrogenation. Whether this is of physiological significance for the formation of acetylcholine can not be decided at present and the problem requires further investigation.

#### SUMMARY

1 The distribution of cytochrome oxidase in nerve tissue of the squid has been studied. In the head ganglion the concentration is remarkably high, the  $Q_{10}$  at  $23^\circ C$  being  $-9.0$  to  $-13.0$ . In the axoplasm extruded from the trunk containing the giant axon the concentration is lower, but relatively high compared with that of the remaining tissue. This finding is evidence for the previous assumption that the bulk of the respiratory enzymes is confined to the axoplasm while in contrast practically all of the choline esterase is found at the neuronal surface.

2 Oxidation of pyruvic acid in the minced head ganglion occurs at a rate similar to that of p-phenylenediamine, the  $Q_{10}$  being about  $-7.0$  to  $-9.0$ . On the other hand, in a ground suspension of the head ganglion the activity of pyruvic oxidase falls off rapidly even if the following substances known to be of consequence in pyruvic acid oxidation are added: cytochrome c, adenosinetriphosphate, diphosphothiamin, diphosphopyridine nucleotide and succinate. In the axoplasm the activity of pyruvic oxidase was small, although in the whole trunk if minced, the rate of  $O_2$  uptake is about the same with pyruvate as with p-phenylenediamine. The axoplasm dissolves rapidly in isotonic solution. Therefore the low  $Q_{10}$  values must be attributed to the rapid loss of activity if the cell structure is destroyed.

3 Pyruvic dehydrogenation is strongly increased in the presence of diphosphothiamin. In view of the concentration of this coenzyme at the neuronal surface this incomplete breakdown, yielding acetic acid and carbon dioxide, may be of significance for the formation of acetylcholine.

rate of  $\text{CO}_2$  production is about 3 times as high as without this compound. The concentration of diphosphothiamin was  $50 \mu\text{g}$  per cc, a concentration not far from the possible or even probable physiological concentration at or near the neuronal surface.

The presence of adenosinetriphosphate has only a slight effect, as shown in the experiment presented in Fig. 4.

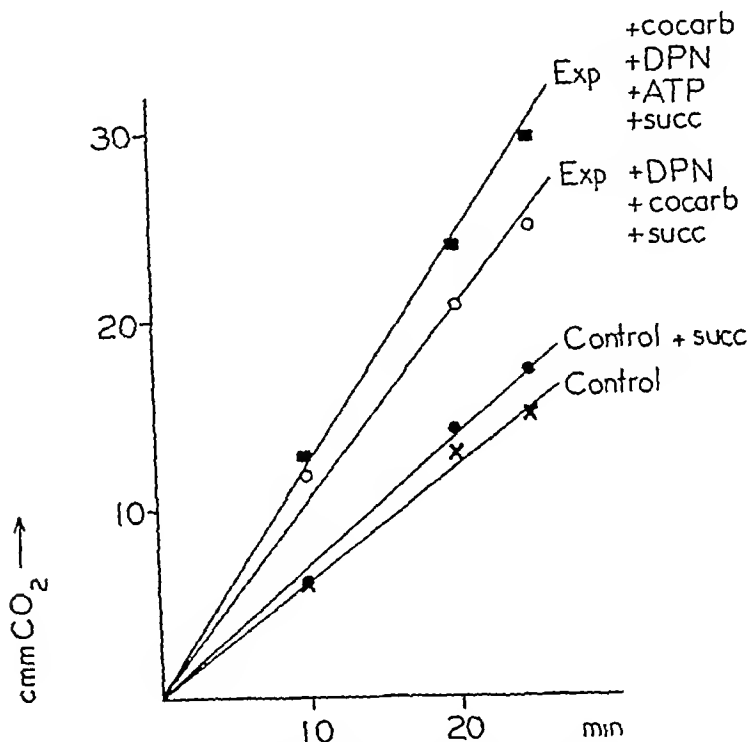


FIG. 4 Effect of adenosinetriphosphate (ATP) on pyruvate dehydrogenation with an homogenized suspension of the head ganglion of the squid. Concentrations used are the same as in the experiment shown in Fig. 3.

### DISCUSSION

The observations here described on the distribution of cytochrome oxidase demonstrate its high concentration in the axoplasm of the nerve fiber as compared with the low concentration in the nerve sheath. Since the physiological activation of oxygen occurs through the cytochrome-cytochrome oxidase system, this distribution appears significant. It does not indicate any direct or immediate connection between oxidation and the action potential, although oxidation must be the final energy source as for all cell processes. The localization of choline esterase at the neuronal surface in contrast to that of respiratory enzymes becomes even more significant. It is worthy of note that the rate of  $\text{O}_2$  uptake is, in absolute values, low even in





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radiation and cerebral cortex, particularly with reference to the differential rates at which these structures can be reactivated from the periphery

#### METHOD

The results are based upon experiments carried out on 9 adult monkeys (*Macaca mulatta*). \*The majority of these animals had had operations on the internal ear through the mastoid bone several weeks before our acute experiment

The technique and apparatus for securing simultaneous electroencephalograms and a record of photic stimulation have been described previously (8, 9). After anesthetizing the animals with nembutal administered intraperitoneally, the scalp was reflected and the calvarium removed from the occipital lobe. The dura was opened and reflected medially. The animal was then placed on a board facing the source of light. In most experiments both pupillary and accommodative reflexes were eliminated by installation of 0.5 per cent solution of scopolamine hydrochloride into the conjunctival sacs of both eyes. This, however, proved to be unnecessary, and satisfactory results were obtained with undilated pupils. Blinking movements were eliminated by continuous use of lid retractors, this procedure was also found to be superfluous. An Adrian-Bronk concentric dipolar needle electrode was passed manually through the middle portion of the first temporal convolution into the lateral geniculate body on one or both sides. When the activity of the tectum mesencephali was studied, a needle electrode was inserted along the tentorium cerebelli. A similar needle electrode was inserted into the optic nerve by passing it through the inner canthus of the eye along the medial margin of the orbit until it reached the optic nerve.

Most of the recording was done by means of a three-channel ink-writing electroencephalograph. For greater accuracy in determining time relations, additional recording was made on bromide paper with a moving loop oscillograph.

The position of the electrodes was controlled anatomically by removing the brain and sectioning it in slabs 1 mm. in thickness after formalin fixation.

#### RESULTS

The results will be discussed under the headings of the individual anatomical structures.

##### 1. Cerebral cortex

From leads placed upon the striate cortex on either the medial or lateral surface of the cerebral hemisphere electrical responses were obtained from photic stimulation of the retina. Leads from any other cortical area have not given such responses, even when placed on the parastriate area there were no responses. The response may be of several types. At slower speeds of stimulation (up to 8-9 per sec.) usually the response consists of two waves, one following the "on" signal and the other the "off". At slow speeds the "on" response is usually greater than the "off" response. But as the speed increases the two may become of equal amplitude (Fig. 1E). With still further acceleration the "off" effect drops out and the resultant effect is a series of approximately sinusoidal waves corresponding to the rate of stimulation (Fig. 1).

*Effect of frequency.* The cortical rhythm may be driven up to a frequency of 34 per sec. Above this level it proved impossible to drive the cortical potentials with the luminous intensities used. Possibly with higher intensities the cortex would respond to individual flashes at higher frequencies. The highest intensity at the eye of the monkey has been 30 foot candles,

\* It is a pleasure to acknowledge the kindness of Dr. John R. Lindsay in placing these animals at our disposal for terminal experimentation.

# MECHANISM OF TEMPORAL FUSION EFFECT OF PHOTIC STIMULATION ON ELECTRICAL ACTIVITY OF VISUAL STRUCTURES

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## INTRODUCTION

IN CONTRAST with its high resolving power in space, the resolving power in time of the mammalian visual system is relatively low. Thus the range of frequencies of intermittent light that man can distinguish from steady light is from 4 or 5 cycles per sec (c p s) at low intensities to about 55 c p s at high intensities (11). Since it is well known that sensory nerves can transmit discrete impulses at rates above this upper limit, it has come to be rather generally assumed, in the absence of experimental evidence to the contrary, that the limiting factor in temporal resolving power must be the retina. Thus "retinal lag" or "persistence" has long been made the basis of many visual phenomena from the fusion (freedom from flicker) of motion pictures when projected at 15 to 20 c p s to chromatic after-images. The essentially retinal locus of the fusion mechanism has, moreover, gained substantial support in recent years from the demonstration by Hecht and his co-workers that Talbot's Law, governing the apparent brightness of intermittent light at the point of fusion, may be derived from the equations of a reversible photochemical reaction, which had previously been evolved to describe certain photosensory behavior of the clam, *Mya arenaria* (10, 11, 12).

It would seem to be a useful working hypothesis that the primary phenomena of fusion are dependent upon the maximum rate at which the *slowest* element in the visual system can be reactivated from the periphery. Such an hypothesis not only permits a relatively direct experimental approach but does not obviate the possible operation of secondary mechanisms such as may be required, for example, to account for such phenomena of flicker constancy as described by Bartley (3).

As one step in our analysis of this problem, experiments have been carried out on monkey, whose fusion mechanism is known to have several important features in common with that of man (6, 13).

In previous papers we have shown that in line with the observations of Bartley (1, 4) and Bishop (5) on cat and rabbit, the electrical activity recorded from the region of the occipital lobe of monkey may be modified in certain characteristic ways by intermittent photic stimulation (8, 9). The present report extends this investigation to the effects of such stimulation on the optic nerve, lateral geniculate body, tectum mesencephalon, optic

frequency of 62 per sec. Higher photic intensities cause augmentation of the response obtained from the optic nerve (Fig. 3)

*Tectum mesencephali* With needle electrodes in the tectum mesencephali (superior colliculus) good driving responses to photic stimulation were obtained. The amplitude of these responses is not so great as that from the lateral geniculate body. That it is not an artefact due to pick-up of spread is evident from the fact that no responses occur when the electrode is placed in the adjacent corpus callosum.

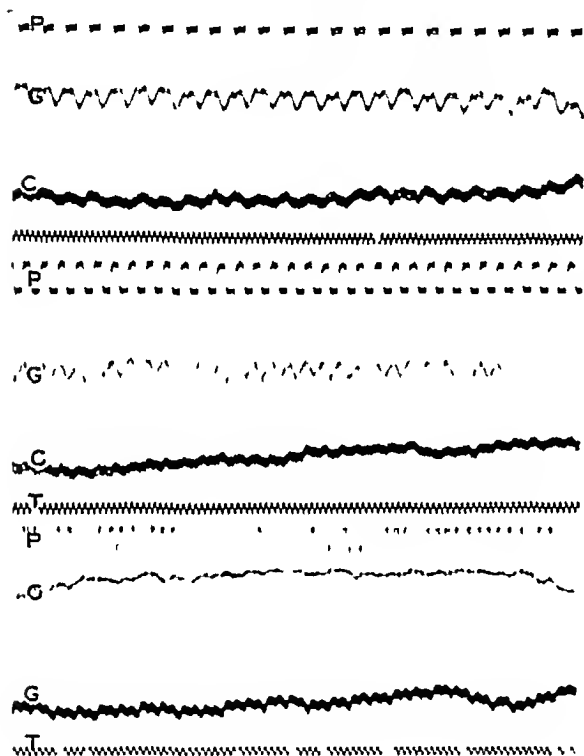


FIG. 2. A series of records from the striate cortex (C) and the lateral geniculate body (G) to show the driving effect at different frequencies. The top record is at 23 c.p.s., the middle at 32.5 c.p.s., and the lowest one at 59 c.p.s. In the first two records both the cortex and lateral geniculate body respond to the photic stimuli (P), but in the third record the cortex has assumed its own rhythm and only the lateral geniculate body responds. At the bottom of each record is a time signal (T) indicating 1/100 sec. The tracings were made on bromide paper with the loop oscillograph.

*Optic radiation* On a number of occasions we have attempted to place a needle in the optic radiations but the responses have never been satisfactory. Whether this is because the fibers of the radiation are more diffuse than at the geniculate level or is the result of the type of impulse passing over these fibers is not evident at this time.

## DISCUSSION

*Localization of driving effect* Evidence of cortical localization of the driving effect was revealed by the fact that driving at rates up to 34 c.p.s. could be obtained only when the leads were located on the striate cortex. No driving could be elicited from the parastriate area (cf. 15, 16). Further

which, using an episcatistor having a light dark ratio of 1 2 means that the effective flicker intensity was 10 foot candles

At a low light intensity it was possible to produce driving with low frequencies whereas high frequencies were ineffective. Thus in one preparation using an intensity of 4-foot candles driving could be obtained at a frequency of 8 but not of 17 per sec. Increasing the intensity at the latter frequency produced good driving.

*Intensity* The importance of the intensity of the photic stimulus is indicated by the fact that in general the higher the frequency, the more intense the stimulus required. We have obtained driving at an intensity below  $\frac{1}{2}$  foot candle. With low intensities driving resulted with low frequencies. The higher ranges of intensity have not been explored.

## 2 Lateral geniculate body

Electrical responses in the lateral geniculate body follow regularly upon photic stimulation of the retina at frequencies up to 59.1 per sec. Beyond this point driving could not be elicited, although the activity of the lateral geniculate body is distinctly changed from that of the resting state. The responses are usually spike-like but at times assume wave form. At times a frequency in the geniculate develops at twice the rate of retinal stimulation, but more often and especially with higher frequencies the geniculate rate is half that of the photic stimulation (Fig. 2).

The lateral geniculate response occurs to minimal intensities of light but is more pronounced to brighter photic stimuli.

## 3 Optic nerve

Responses obtained from the optic nerve are in many respects similar to those from the lateral geniculate body. They will follow stimuli up to a

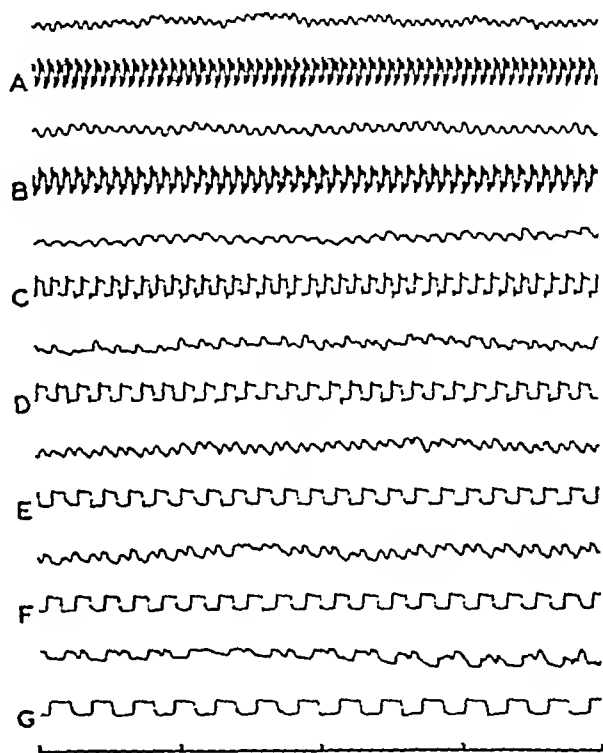


FIG. 1. A series of electrocorticograms of the striate area showing the driving effect of photic stimulation of the retina at frequencies from 3.5 cycles per sec (G) to 15 per sec (A). The distinct "off" and "on" effect disappears between 7.5 c.p.s. (D) and 9.5 c.p.s. (C).

second *below* that for man. If the velocity of a reversible photochemical reaction in the retina (as suggested by Hecht, 11) establishes a maximum limit beyond which fusion occurs, it seems that *supramaximal* values would be required for driving the optic nerve and lateral geniculate body at a rate of 62 and 59 c p s respectively.

*Possible pupillary effect eliminated* Most of the determinations of maximum fusion frequency in man have employed either an artificial or the natural pupil. However, Halstead (7) could demonstrate no significant difference in critical fusion frequency in a normal subject with maximal pupillary dilatation produced by scopolamine. As mentioned previously in our statement of method, mydriasis had no influence on the driving effect in monkey.

*Other evidence of neural basis of fusion* Recently reported observations by Kluver (14) indicate that, in contrast with the behavior of a normal monkey, the differential response of a monkey in which both occipital lobes have been removed is not disturbed by substituting an intermittent light of moderate intensity for a continuous one. This was true when the intermittent rate was as slow as 4 c p s (light to dark ratio =  $\frac{1}{3}$ ). This might be due to marked shift or lowering of critical fusion frequency associated with removal of the occipital lobes. As support for such an interpretation, however, it should be proved to be impossible to establish a differential response in the monkey lacking both occipital lobes to two stimuli equated as to luminous flux but differing in rate of intermittency, for example, 10 c p s versus 5 c p s.

The observations of Seitz (17) that strychnine applied to one eye of human subjects raises the critical fusion frequency for that eye without affecting the other eye would seem to be in line with a neural interpretation of the fusion mechanism, unless it can be shown that strychnine markedly augments photochemical activity. In Seitz' experiments the elevation of critical fusion frequency under strychnine was of the same order as the depressing effect on fusion frequency of acute exposure to the anoxia associated with a simulated altitude of 20,000 feet. It should perhaps be noted that his conclusion that the effect of strychnine "cancels" the effect of anoxia is somewhat misleading. That it may *mask* the effect of anoxia on critical fusion frequency is probably as much as can be concluded from his results.

#### SUMMARY

The driving effect of intermittent photic stimulation on the electrical activity of the optic nerve, lateral geniculate body, tectum mesencephalon, optic radiations and cortex has been explored in monkey (*Macaca mulatta*). It was found

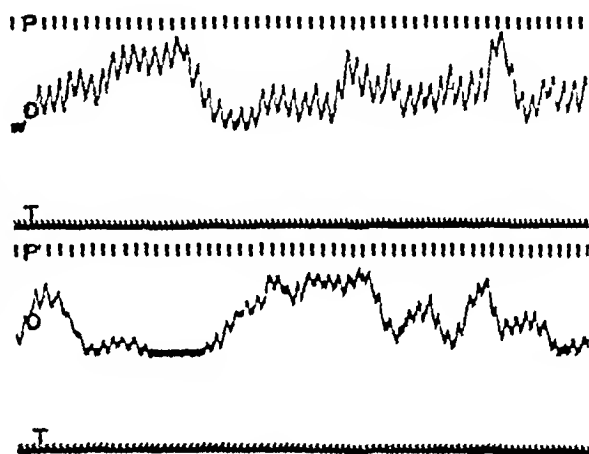
(1) At an intensity of 10 fc, the optic nerve and lateral geniculate body could be driven at a maximum rate of 62 c p s, and 59 c p s respectively, a

investigation suggests that photic stimuli may modify the activity of areas other than the striate cortex. This point is now being studied in more detail.

*Effect of intensity* The effect of intensity, is in line with our previous findings based upon recording from leads attached to the scalp of the unanesthetized monkey (8, 9). However, the range of frequencies through which intensity is effective is greater in the present instance where recording was from leads placed directly in contact with particular brain structures and with the animal maintained under relatively deep anaesthesia than in the previous experiments.

*Differential driving rates for a given intensity* The most striking feature of the present results lies in the marked difference at which, for a given in-

FIG. 3 Two records taken from the optic nerve (O) showing the response to individual photic stimuli (P) at a frequency of 54.2 (above) and 62 (below) per sec. A time signal (T) indicating 1/100 sec is at the bottom of each record. Both tracings were made on bromide paper with a loop oscillograph.



tensity of light, the various visual structures could be reactivated (driven) from the periphery. With a luminous flux (at the eye of the animal) of 10 fc the optic nerve and lateral geniculate body drove regularly throughout a range of from 1 to 62 c p s and 1 to 59 c p s respectively (2). The striate cortex on the other hand, followed regularly at 1 c p s but could never be driven above 34 c p s. Since this latter value falls within the range at which Brecher (6) found intermittent light to *fuse* for monkey, it is possible that a fusion mechanism provided by the cortex constitutes a physiological barrier to driving at higher frequencies. Whether this is definitely the case must await further investigation since it is possible that the nembutal anaesthesia, which we employed, acted to suppress the driving rate of cortical structures while sparing, relatively at least, more peripheral structures. Also, a satisfactory driving effect from the optic radiations has not yet been obtained. It is significant, however, that driving of the optic nerve and lateral geniculate body occurred at a rate as high as 62 and 59 c p s respectively. This is well above the maximum values for fusion, found by Hecht and Verrijp in man (11). Furthermore, Brecher's results indicate that for the range of intensities employed by us, fusion occurs for the monkey at several cycles per





rate that is well above the maximal critical fusion frequency for man and probably for monkey,

(ii) The cortex of the striate area could be driven at a maximum rate of 34 c p s

(iii) While a driving effect could be obtained from the tectum mesencephali and from the optic radiations, it was not sufficiently stable to permit a determination of maximum rate

(iv) The possibility is considered that our findings indicate a fusion mechanism in the cortex, which limits the temporal resolving power of the primate visual system

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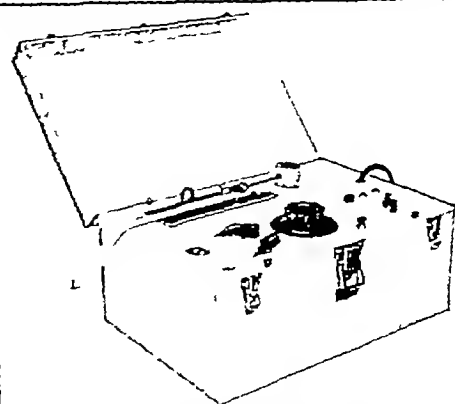
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### AN APPRECIATION

STEPHEN WALTER RANSON, Professor of Neurology and Director of the Institute of Neurology of the Northwestern University Medical School, was a distinguished contributor to anatomical and physiological science during the past four decades. After receiving his baccalaureate at the University of Minnesota at the age of 22, he, in the next five years received successively the M S, Ph D and the M D. After two further years of academic and medical apprenticeship he commenced his academic career at Northwestern Medical School in 1909 and three years later was appointed to the chair of anatomy. In 1928 he was selected to direct the newly founded Institute of Neurology.

His life was devoted to the study of the minute and barely attainable, and he discovered where others have looked and not beheld. Like the astronomer who knows the existence of a star before it is seen, he early deduced the existence of a system of nerve fibers which had escaped the microscope, and by the development of special stains brought them into view. To the study of these fine nerve fibers he, and others returned again and again, until their connection with pains that have baffled the physician and surgeon is finally becoming clear.

In his recent years he addressed himself to the part of the nervous system, minute and deeply concealed in the brain, which controls the functions of the human body on which life depends. To this he brought a device which enabled him to penetrate deeply into the substance of the brain, to stimulate or to destroy, and to chart with the accuracy of the surveyor the functional topography of the brain. The closeness of his search and the exactness of technique was again rewarded by disclosing the function of another system of nerve fibers—fibers which pass directly from the brain to the pituitary gland and control its secretion.

T C R

muscles To avoid these contractions it is sufficient to wash the nerves frequently with Ringer's solution, and the skin of the legs with fresh water In these experiments mechanical excitations of feet and legs were employed After a certain time, when the reflexes are weakening, the excitations must be repeated and summated In this period, one single excitation can be inefficient, while repeated excitations still produce strong reflexes Disappearance of reflexes is established when the strongest repeated excitations do not provoke any reaction

## RESULTS

*Spontaneous movements* In experiments with American frogs, preparations of isolated spinal cord and legs frequently show spontaneous move-

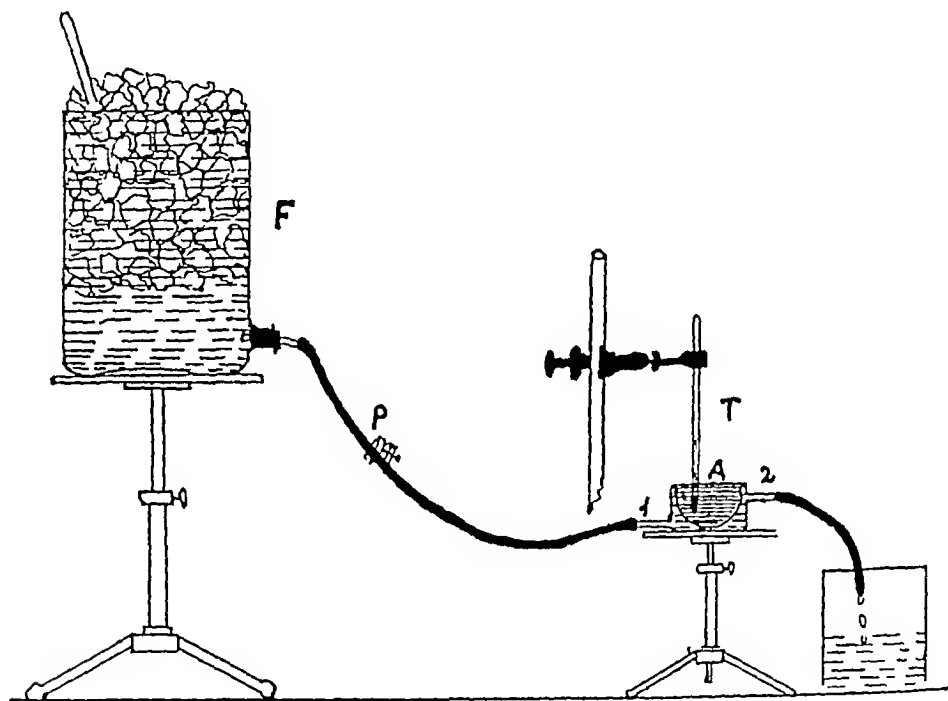


FIG 1

ments These movements are well coordinated, and the legs flex and extend alternately Such reactions can be observed for a few minutes In some cases, just one leg presents this form of activity while the other one is at rest Spontaneous coordinated movements are not to be seen when the spinal medulla is in a Ringer's bath at low or high temperatures, below  $3.5^{\circ}$  or above  $21^{\circ}\text{C}$  In many cases high temperatures produce strong excitations the legs react in discoordinated movements that soon disappear Between  $8^{\circ}$  and  $18^{\circ}\text{C}$  spontaneous movements are produced in almost all cases Perhaps, the spinal cord at these temperatures finds itself in a state of increased excitability and is able to answer to excitations not directly made, but that are due to exposure of the tissues to air

*Alterations of reflexes* In American frogs the reflexes do not show all the

# TEMPERATURE EFFECTS ON REFLEXES OF ISOLATED SPINAL CORD

## HEAT PARALYSIS AND COLD PARALYSIS

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EFFECTS of temperature on reflexes of frogs have been studied in experiments in which temperature changes affected the entire body. No experiments are recorded in which the influence of temperature has been confined to the nervous system without affecting muscles and receptors. Employing a preparation of the vertebral column containing the spinal cord isolated from the body, except by the iliac nerves which maintain nervous communication with the legs, and by putting the vertebral column in baths of Ringer's solution at different temperatures, it has been shown in Brazilian frogs (*Leptodactylus ocellatus*), that the form and also resistance of the reflexes present constant alterations at temperatures above and below an average temperature of about 18°C. It was also found that the curves of resistance at different temperatures show quantitative variations when obtained in different seasons of the year (1).

In this paper are related the results of similar experiments in American frogs (*Rana pipiens*) conducted a year ago at Yale University. Brazilian frogs develop convulsive attacks if the spinal cord is cooled below 8.5°C. This fact does not permit experiments on the reflexes at these low temperatures. However, in American as in European frogs, this attack is normally produced only at temperatures below 0°C. Using *Rana pipiens* it has now been possible to enlarge the limits of temperatures employed as it happened before in the experiments conducted at Paris.

### APPARATUS AND METHODS

The preparation is made just before each experiment. After separation of the spinal cord from the superior centres, the animal is pinned as usual on a cork plate. The skin is cut along the dorsal median line. After cutting the aponeuroses, the urostyle is picked up with the forceps and the sacro-coccygeal and ilio-coccygeal muscles severed. The lumbar nerves are dissected and isolated. It is then possible to separate the vertebral column from the body. The legs with the isolated iliac bones are attached by threads to a horizontal support if the experiment is intended to be a simple observation, or on the plate of a myograph if records are wanted. Figure 1 shows schematically the experimental procedure to obtain Ringer's baths at different temperatures. The essential part is the small apparatus A. The Ringer's solution in the upper part is isolated from the circulating liquid (water; or water, ice and salt for low temperatures) coming from flask F. Careful regulation of the output and of the temperature of the water permits having the Ringer's baths at the desired temperature.

In experiments conducted during the winter in New Haven, the laboratory's atmosphere was too dry. Nerves in contact with the rim of the bowl containing the Ringer's bath were soon excited by Ringer's solution which had previously overflowed and by losing water, had become a hypertonic solution. This happens particularly in experiments at high temperatures. The nerve's excitation is shown by spontaneous contractions of the leg.



the communication with the flask F is opened and the circulation of cold water produces a fall of temperature so that the oil attains  $20^{\circ}$  in 4 or 5 minutes. The reflexes return with their normal characteristics. Therefore, the spinal cord, which has remained in the oil without contact with the air, is paralysed by heat and recovers from this paralysis only by cooling.

*Resistance of reflexes* The time needed for the disappearance of the re-

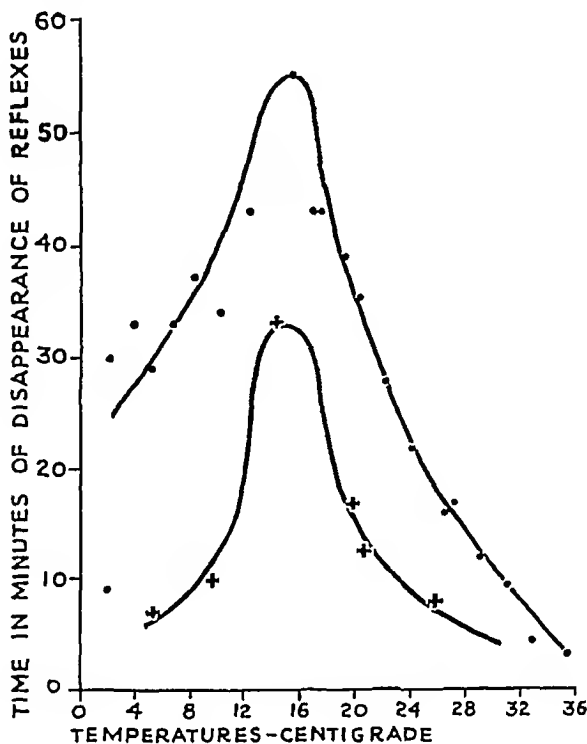


FIG. 2. Curves of the time of disappearance of the reflexes when the isolated medulla is submitted to different temperatures. Plus signs, experiments made in January, black dots, experiments made in February and March.

flexes is variable with the temperature, and the curves obtained are of similar shape as those previously obtained in Rio de Janeiro and Paris. However, the curves are themselves not superimposable, and in this case, it seems that the action of climate and general conditions of the life of the frogs must be taken into consideration. In the experiments at New Haven, two different curves were obtained (Fig. 2), the first in January at the beginning of the cold weather, and another between February 10 and March 5. The experimental results are reproduced in Table 1 for the first period and Table 2 for the second.

The time before the disappearance of the reflexes increases with increasing temperatures attaining a maximum at  $15^{\circ}\text{C}$  on the average, and

alterations previously observed in Brazilian frogs. However, at low temperatures the same type of alterations are to be observed: flexion is prolonged and the flexed legs show rapid alternate movements in the different segments, it is a type of clonus produced by cold. However, on the other hand, at high temperature the reversal of reflexes observed in Brazilian frogs, consisting of a change of flexion into active extension, is not seen in American frogs. The reflexes maintain the flexion type until they disappear. In some cases, after flexion, the return of the legs to a normal position is not simply due to relaxation of flexor muscles but to a slight contraction of the extensors.

*Exhaustion of reflexes—Heat paralysis and cold paralysis* If the spinal cord is subjected to different temperatures, the reflexes disappear after different time intervals, in direct correlation with the temperature. If the spinal medulla is again put in a Ringer's bath at room temperature immediately after cessation of the activity of the spinal centres under direct action of cold or heat, two different results can be obtained. When in the first part of the experiment, the temperature of the bath is between  $14^{\circ}$  and  $29^{\circ}\text{C}$ , the reflexes do not return: the spinal centres are exhausted. When the previous temperature is below  $14^{\circ}$  or above  $29^{\circ}\text{C}$ , the reflexes return normally. Under the action of cold or heat, the lack of action of the medullary centres is not due to exhaustion. The nervous centres are paralysed, and they can present a *heat paralysis* or a *cold paralysis*. Alternate periods of paralysis and activity can be obtained many times in the same preparation. The bath's temperature where paralysis is produced must be sufficiently low or sufficiently high so that the state of paralysis is attained in a short time. After some repetitions of the state of paralysis and recovery, the reflexes are definitively exhausted. For example, in one experiment (Feb 6), in the Ringer's bath at  $35^{\circ}$ , the medulla lost all reactions after 3 min; when removed to another bath at  $20^{\circ}$ , the reflexes were present again 30 sec afterwards; a second period of temperature at  $35^{\circ}$  produced the disappearance of reflexes after 1 min. These reflexes reappeared after the preparation was replaced in the bath at  $20^{\circ}$ . Finally, the third time that the medulla was subjected to  $35^{\circ}$ , the reflexes disappeared permanently.

Heat paralysis of the nervous centres has been studied by different methods by other physiologists. Working with the whole frog, Winterstein (2) explained this phenomenon as the result of an asphyxiation of the centres. The results obtained in experiments with the isolated spinal cord do not support this explanation. Control experiments demonstrate that the restoration of reflexes, after the return of the paralysed medulla to normal temperature, is obtained without the contact of the nervous centres with the outside air.

Instead of Ringer's solution, the apparatus for cooling the medulla contained paraffin oil, which is heated to a temperature of  $35^{\circ}\text{C}$  by the circulation of hot water in the external part of the bowl. Then the communication of the bowl with the flask F is closed and the hot water is replaced by cold water. The oil's temperature is maintained for the necessary time. Putting the medulla of a preparation in the hot oil, after the reflexes have disappeared

*Localisation of action of heat* Heat paralysis, observed in other ways, has raised the question Does paralysis result from the action of temperature on the nervous centres, or on the peripheral organs? Experiments of Archangelsky and of Becht (4) have shown that it is the central nervous system that loses its functions under action of high temperatures In the experiments here reported, the question is more limited The muscles and skin receptors are not subjected to heating, and the results can be due only to the alteration of the temperature of the nervous centres or the peripheral nerves

The analysis of this point has been made in several different experiments The nerve trunks of the preparation were enveloped with cotton, while the vertebral column and the legs were at room temperature Between the two nerve bundles, and also contained in the cotton envelope was placed one thermometer The cotton was then soaked with a hot Ringer's solution which was frequently renewed, and in this manner, the temperature was maintained between 32° and 34°C The reflexes disappeared after 16 min At this temperature the whole spinal medulla does not react after 4 to 6 min Disappearance of the reflexes, due to nerve paralysis, takes then three times longer to be produced than central nervous paralysis In other experiments the two legs of the preparation were separated by a section along the median line While one leg was in hot Ringer's bath, the other and the medulla were outside the apparatus at room temperature For example, in one experiment, in the leg heated at 34.5°C in the bath, the reflexes disappeared after 17 min, while in the other leg they were perfectly normal and strong At 34.5°C, the spinal cord loses its functions after 2.5 or 3.5 min In this experiment, the time necessary for paralysis due to the action of the temperature on the peripheral organs, including the nerves, was 5 to 6 times greater than in the case of central paralysis

*Recovery time after heat or cold paralysis* After cold or heat paralysis, the spinal cord being again replaced in baths at room temperature the time intervals during which the reflexes reappear are not constant In the case of cold paralysis, the recovery time decreases when previous temperature producing paralysis increases, in the case of heat paralysis, the recovery time increases with increasing temperatures Figures of values obtained are found in column 5 of Table 2

## DISCUSSION

In the conditions of the experiments reported, the spinal cord is contained in the vertebral canal without blood circulation and covered by a thick envelope of tissue Respiratory exchanges between the nervous centres and the outside air may be completely suppressed The medullary centres can maintain their functions during the time they dispose of a certain amount of oxygen previously existent, or during the time that carbon dioxide or other substances produced by metabolism do not attain a level where paralyzing action manifests itself

Between 14° and 29°C the nervous centres' function disappears when

Table 1 Time of disappearance of the reflexes in different temperatures, January 13

Temperatures of the Ringer's baths ( $\theta$ ) Centigrade	5 5	9 5	14 0	20 5	19 7	25 8
Time of disappearance of the reflexes ( $t_1$ ) in minutes	7	10	33	12,5	17	8

Table 2 Time of disappearance ( $t_1$ ) of the reflexes in different temperatures of Ringer's baths ( $\theta$ ), and recovery time ( $t_2$ ) in baths at room temperature ( $\theta'$ ) The time of exhaustion at room temperature when the medulla is put from the beginning at the Ringer's bath ( $\theta'$ ) is  $t'$ 

1	2	3	4	5	6
$\theta$	$\theta'$	$t'$	$t_1$	$t_2$	$1 \frac{t_1}{t'}$
2,1	17,0	43	9	28	0,35
2,1	16,5	43	30	36	0,16
3 8	16,5	43	33	30	0,30
5,1	17 0	43	29	26	0,39
6 6	16,8	43	33	26	0,39
8,1	17,0	43	37	23	0,47
10 0	14,7	55	34	12	0,79
12,0	15,0	55	43	5	0,91
14 9	14 9	55	55	0	1,00
16 7	16,7	43	43	0	1,00
17 0	17,0	43	43	0	1,00
19 0	19,0	39	39	0	1,00
20,1	20,1	35	35	0	1,00
22 1	13,8	55	28	0	1,00
24 0	14,8	55	22	0	1 00
26,3	14 9	55	16	0	1,00
27 1	19 0	39	17	0	1,00
29 0	18,0	40	12	5	0 87
31 0	18 9	39	9,5	8,5	0 78
33 1	19,0	39	4,5	20,5	0 48
35,0	17,9	40	3 5	23	0,42

decreasing afterwards with higher temperatures. In these experiments results at high temperatures were more regular than at low. The results were not obtained the same day, but this does not explain the irregularities.

In the middle temperatures the time of resistance is considerable and the question arises whether, in the end, the exhaustion of the reflexes is truly due to an exhaustion of the nerves or nervous centres, or if the muscles deprived of circulation at room temperature are no longer able to react. New experiments demonstrate that this is not the case. Sometimes the total time of resistance of the reflexes is greater than the maximum observed in the curve, if the spinal cord is subjected to a low temperature before and to the middle temperature afterwards. In control experiments all circulation in the posterior half of the body was tied, while the anterior half was in a nearly normal condition. Reflexes were present more than an hour. Finally, Baghioni (3) in experiments with his preparation of spinal medulla isolated and exposed to air, and communicating by the sciatic nerve with the leg cut at level of the knee, has seen that at room temperature the reflexes resist more than two hours.

5 Reflex exhaustion is permanent when the spinal cord is subjected to temperatures between  $14^{\circ}$  and  $29^{\circ}\text{C}$

6 Above  $29^{\circ}\text{C}$ , before exhaustion, the spinal centres are paralysed after intervals of time decreasing with increasing temperatures. Recovery is obtained if the spinal medulla is put again in the mean temperature. Heat paralysis is not due to asphyxia, and recovery does not depend of any exchange between nervous centres and the outside atmosphere

7 Below  $14^{\circ}\text{C}$ , cold paralysis is observed and the time for it to be produced is shorter the lower is the acting temperature. Recovery also is obtained, after paralysis, when the medulla is placed again in the mean temperature. Cold paralysis, as heat paralysis, intervenes before the exhaustion of the centres

8 Heat and cold paralysis are not due to an interruption of conduction in the nerve or to any action on peripheral organs. Under the action of low or high temperatures the nervous centres cease their functions long before peripheral nerves and organs

9 The time of disappearance and the time of resistance of the reflexes after recovery from paralysis when the spinal cord is again at room temperatures may give available indications upon conditions of nervous centres at the moment when cold and heat paralysis are produced. At high and low temperatures, nervous centres are paralysed by concentrations of metabolic products, which are ineffective in mean temperatures. The intensity of action of these products is dependent on the temperature

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complete exhaustion is attained. Below  $15^{\circ}$  and above  $29^{\circ}\text{C}$ , nervous centres are paralysed at a moment when they dispose of the reserves, that are able to permit restoration of functions in a normal manner if they are placed again in room temperature. The length of this second period, during which the reflexes recover after a previous paralysis, varies directly with the deviation of the temperature of the paralysing bath from the average. This suggests that the action of this lack of oxygen or of the products of metabolism is not exerted at the same level when the temperatures are different, in the cases of temperatures that produce cold or heat paralysis. These levels decrease with decreasing temperature in the case of cold paralysis, and increase with increasing temperature in the case of heat paralysis.

Taking the metabolism's level attained at average temperatures, when reflexes disappear by exhaustion, as unity, immediately after cold or heat paralysis, the nervous centres have a reserve that is given by the relation  $t_2/t'$  ( $t_2$  recovery time,  $t'$  time of exhaustion of the reflexes at room temperature  $\theta'$ ). At this moment the level attained by the products of metabolism is  $1 - t_2/t'$ . In column 6 of Table 2 are reported the values of this difference. Plotted on rectangular coordinates, these values form nearly an ascendent straight line for low temperatures, and a descendent straight line for high temperatures.

On the ground that intensity of the tissue metabolism increases when temperature increases, it could be expected that the period of the resistance of the reflexes is greater the lower the temperature. As a matter of fact, experimental curves have not confirmed this assumption, they present one maximum, and below a given temperature the curve descends. This fall can be well explained otherwise than by an alteration of the law of the variation of metabolism in function of the temperature. Paralysis by cold intervenes at a moment when the concentration of the metabolic products is lower than the level for exhaustion of the centres when these are under action of mean temperatures, and this paralysing concentration is decreasing when temperatures decrease.

### CONCLUSIONS

1 In preparations of isolated spinal cord and legs from American frogs, spontaneous coordinated movements are observed if the temperature is between  $3.5^{\circ}$  and  $21^{\circ}\text{C}$ .

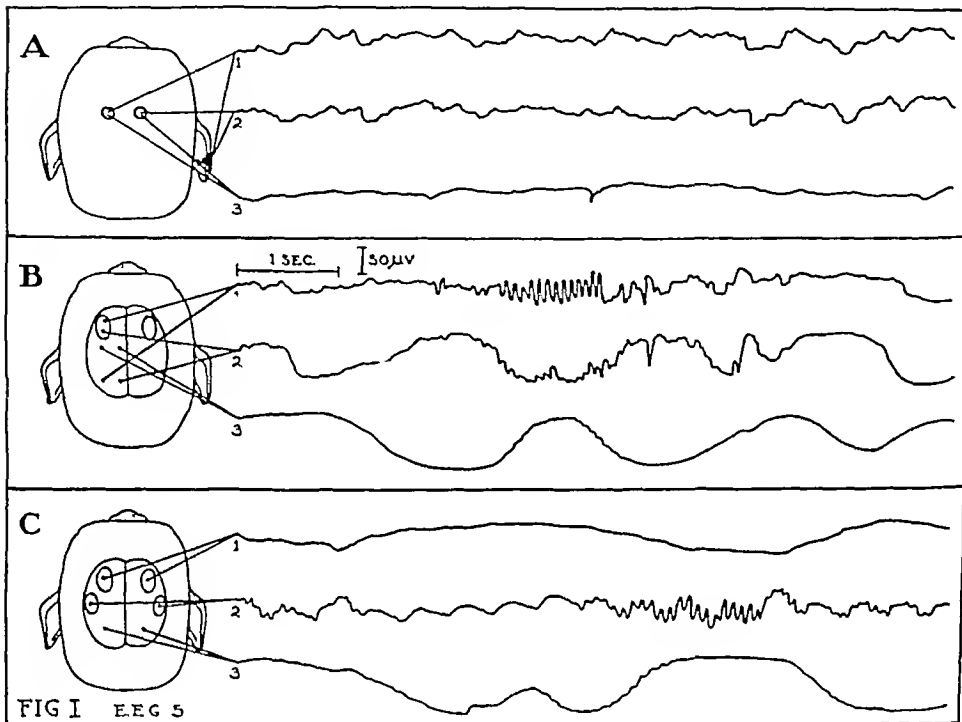
2 Differences are noted between American and Brazilian frogs in the form of reflexes, when the spinal medulla is subjected to different temperatures.

3 The time of resistance of spinal reflexes at different temperatures, in American frogs, as in frogs previously studied (Brazilian and European frogs), form curves with a maximum for a mean temperature. Below and above this temperature the time of resistance decreases.

4 Position and value of this maximum are dependent on habitual temperatures and conditions in which the frogs are living.

Records made through the skull of the anesthetized animal showed characteristic rhythmic potential differences of rather low amplitude but with a rate of 8-10 per sec oscillations such as are normal in these animals (Fig 1)

Next, the bone flap made 4 days previously on the right side was turned back and the electrodes placed on the tissue from which cortex had been previously separated. At this time, before the left basal ganglia were exposed, because this was the first experiment of its kind, various experimental procedures were tried which were subsequently proven



irrelevant. The left skull and dura were then removed and leads placed on the right basal ganglia and right side of thalamus. Later they were replaced so as to record simultaneously from the basal ganglia of both sides (Fig 1). The records thus obtained were unlike those recorded from any other areas and proved later to be characteristic for the caudate and putamen.

From these nuclei the EEGs were of low voltage and displayed a background activity which varied in amplitude but showed a more or less regular rate of oscillation at about 6-8 per second. These resembled in rate the medium frequency waves obtained from cortex but never were complicated by the rapid components found in cortical records.

Superimposed upon the more regular low voltage background activity appeared bursts of much higher amplitude (Fig 1B). These occurred spontaneously and at irregular intervals varying from every two or three seconds to several minutes. Their character was always the same. Regular rapid spikes suddenly appeared from a record showing little activity. The bursts lasted two to five seconds and increased in both amplitude and rate during the middle of the burst, while beginning and end were both lower and slower. Such bursts were suppressed by any manipulation of the surrounding tissues and, apparently, by loud sound or repetitive light flashes.

In Fig 1B one such burst is shown. It is recorded strongly from caudate leads (1), less strongly from the putamen (2). In Fig 1C only the putamen of one side is active.

Records from the 3 animals from which these bursts were obtained all showed that

# ELECTROENCEPHALOGRAM OF DECORTICATE MONKEYS\*

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SINCE electrical potentials can be recorded from all active living cells, the pattern of rhythmic potential differences known as the electroencephalogram (EEG) obtained from the complex surface of the brain is probably an algebraical summation of activity within the underlying cells. To understand the nature and composition of this pattern it is therefore of interest to attempt analysis of various anatomical components of the brain which might contribute to its structure.

Recent studies have already given evidence that the subcortical nuclei directly affect the EEG, since, in the monkey, destruction of portions of the basal ganglia causes prompt and permanent changes in the character of the EEG (7, 8), although lesions confined to cortical tissue do not alter it even if an entire hemisphere is removed.

Furthermore, previous investigators had already established the fact that independent rhythms may be obtained from thalamus (11, 1), cerebellum (11), and from the "basal regions" (3, 4, 6). In the present paper are reported the results of further investigation of the interrelationship of cortical and subcortical structures as indicated by their spontaneous electrical activity.

## METHOD

Five monkeys (*Macaca mulatta*) were used. In four of these, records were taken first in the normal and finally in the completely decorticate state. Two were chronic preparations and two acute, as it was thought that perhaps the immediate effects of ablation might affect the EEG. However, the results in all four instances were alike. The fifth animal was hemidecorticate throughout the experiment.

A Grass, three-channel ink-writing oscillograph was used. Bipolar silver wire electrodes were placed directly on the tissue in most instances. At other times records were taken from the intact skull. Occasionally monopolar leads were used, the indifferent electrodes being then attached to both ears. All animals were lightly anesthetized with Dial throughout the procedure (0.6 mg. per kg. body weight, given one-half intraperitoneally and one-half intramuscularly). They remained in an even state of anesthesia until the end of each experiment. The decortications, both acute and chronic, were carried out by the same method and surgical technique.

## EXPERIMENTAL DATA

In the first three experiments the electrical activity of totally decorticate preparations was investigated.

*Expt 1 (EEG 5) Dec 4, 1942.* A large, nearly mature, male monkey weighing 5.0 kg. had had the left cerebral hemisphere removed one year previously on Nov. 11, 1941, for another purpose. EEG records from the skull over the remaining hemisphere had been normal in the interval. Four days before the present experiment the right cerebral hemisphere was also removed. The animal was then kept in an incubator and tube-fed. Its condition was excellent at the time of the experiment.

\* Aided by a grant from the Josiah Macy, Jr. Foundation.



that it was a greater distance from the excitable tissue to the left side of the skull, since the left hemisphere had been removed

Figure 2B is part of a record taken from leads placed directly on the tissue which remained on the left. As verified at autopsy two leads (Fig 2A, 1) were on the caudate and putamen, two (2) were on thalamus, and two (3) remained on the right cortex. It can readily be seen that none of the three areas produced a pattern of EEG which was like that of any other area but that the records from thalamus and cortex are similar and at times synchronous in rate and pattern. From caudate and putamen potential changes were once more obtained which were of low amplitude and fairly regular at a rate of 6 to 8 per sec, without any trace of the rapid component found in electrocorticograms and records from the thalamus

In this experiment records were taken from 9 a m to 1 30 p m from left basal ganglia, while the right cortex remained intact, in an unsuccessful effort to obtain the spontaneous rhythmic bursts of activity seen in the record of the previous animal. The right cortex was then removed. As soon as the electrodes were replaced on the same spots on the left side, spontaneous bursts of activity appeared from caudate and putamen and were present throughout the remaining period of the experiment. They were also obtained from the basal ganglia of the right as shown in Fig 2C

At autopsy there was no remaining cerebral cortical tissue, except a small bit about 4 mm in width just rostral to the optic chiasm in the midline

*Summary* In this animal when the left hemisphere was absent, but the right intact, independent rhythms were recorded simultaneously from the left basal ganglia, the left side of the thalamus and the right cortex. These remained consistently typical for each area and differed from each other over a period of 5 hours. During this interval no spontaneous bursts of spike activity appeared in the left basal ganglia but when the right cortex was removed, the record from the left basal ganglia, and from caudate and putamen on the right acquired spontaneous bursts of spike-like hyperactivity such as were seen in the decorticate preparation of Expt 1. Between bursts the character of the activity from the left basal ganglia was apparently unaltered by the right hemispherectomy

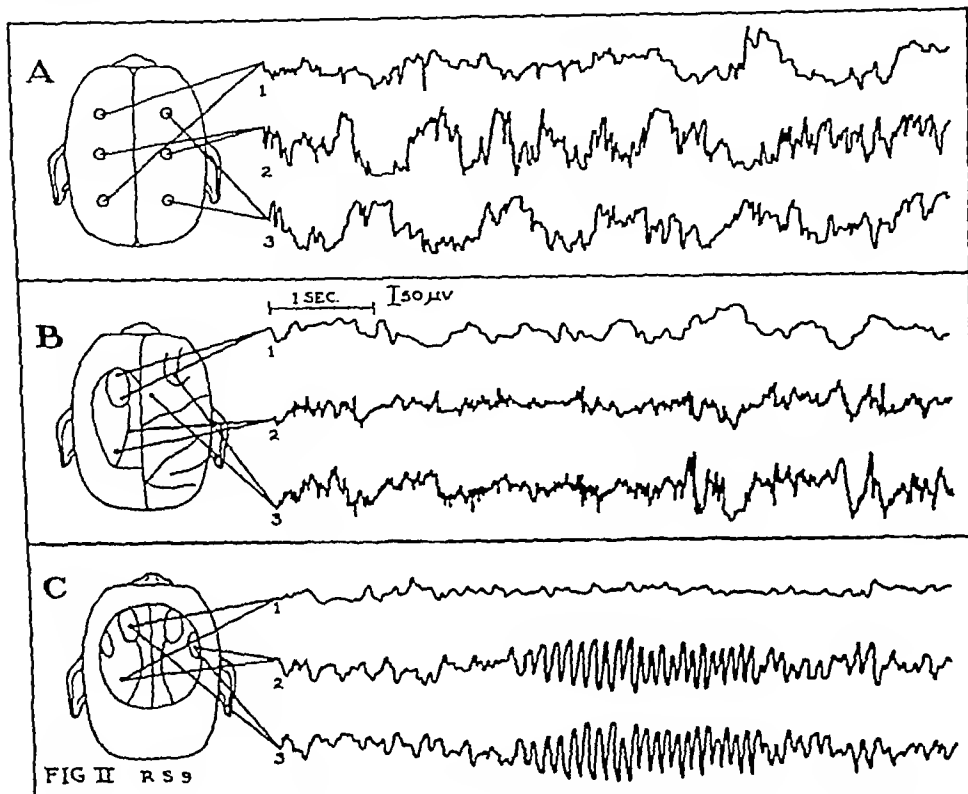
*Expt 3 (EEG, 14), Jan 8, 1943* Under Dial anesthesia the cerebral cortex of this normal macaque (weight 4.3 kg) was exposed bilaterally and normal EEG records obtained from both hemispheres. Following this, area 6 of the left side was gently excavated and electrodes placed on the exposed caudate nucleus. Figure 3A shows the type of record obtained from the left side at this time. Record A1, is from caudate, A2, from area 4 of the cortex about 5 mm from the excavation, and A3, from the postcentral cortex just across the central sulcus from area 4. The excavation of area 6 apparently produced excitation of area 4, but not of the postcentral region for the large slow waves seen in A2 were not present preceding the removal of area 6, but persisted following this operation until the hemisphere was removed. It is to be noted that, in these records, although caudate potentials were not synchronous with either of the two cortical areas, the caudated record differed markedly from that in the decorticate preparation as shown in Fig 3C

Figure 3B is characteristic of records which followed removal of the left cerebral cortex. This record from the left caudate still resembles that of 3A and not that of the same nucleus in 3C. Again, as in A, cortex, caudate and thalamus each presented a different pattern

Next, after removal of the right hemisphere changes such as were found in Expts 1 and 2 appeared. Spontaneous bursts of spike potentials became frequent from the basal ganglia of both sides. Figure 3C shows two such bursts, one from left and one from right caudate which are not, however, simultaneous. Neither of these bursts was transmitted to the thalamus. However, occasionally during such bursts a faint trace of the same pattern appeared in the thalamic records, probably due to spread of impulse

During the remainder of the experiment various lesions of areas surrounding the basal ganglia were made in an attempt to affect the pattern of the record from the basal ganglia. Practically all of the thalamus was removed piecemeal without affecting the bursts. Next the hypothalamus was approached via the midline. Leads placed on hypothalamic tissue in various places at no time recorded any marked pattern in which amplitude and rate could be measured. But excavations here promptly diminished the activity in the EEG from basal ganglia. Since there was then some respiratory difficulty it was felt that these last results were equivocal and conclusions as to the effect of hypothalamus were deferred until the next experiment

they might appear only in one portion (caudate or putamen) of one basal ganglion but that, when marked, the entire complex fired synchronously. The basal ganglia of the two sides did not necessarily fire synchronously however. There was never any evidence of bursts of spontaneous activity in thalamus alone, although characteristic potentials appeared from this area also. Leads placed on internal capsule or corpus callosum near the basal ganglia did not record such bursts.



From the thalamus potentials were recorded which were of lower amplitude. The rate was faster than that of caudate or putamen, its pattern more irregular. There were at times both fast and medium-slow components such as appear in EEGs from cortex.

At autopsy the brain was cut in frontal slices after hardening in formalin. The points upon which the electrodes had been placed were verified. A small bit of cortical tissue near the left insula was all that remained of cortical gray matter.

**Summary.** Rhythmic potential changes were obtained from both sides of the remaining tissue of a monkey which had been decorticate for four days and hemidecorticate for a year. These EEGs, like those of cerebral cortex, had a rate and amplitude characteristic for the area under investigation, but the pattern from basal ganglia differed markedly from that of cortex. The most striking characteristic of the former was spontaneous bursts of spikes appearing at irregular intervals. These were never obtained from thalamus or other sub-cortical structures.

**Expt 2 (R S 9) Dec 11, 1942.** This animal, weighing 5.4 kg, had had its left cerebral hemisphere removed on Nov. 23, 1942. On Dec. 14, 1942, it was anesthetized under Dial and records were taken first from the left and right sides of the skull. Fig. 2A shows such records which are characteristic for normal sleep or anesthesia except that the record from the left side is of lower amplitude than that from the right. This latter merely indicates

(iv) The falx was next removed after ligation of the longitudinal sinus and the right caudate sucked out from beneath the cortical tissue. The pattern of the EEG was then unchanged except for a slight diminution of amplitude. At this time records from thalamus and postcentral cortex were synchronous and similar in pattern.

(v) The left thalamus and then all of the thalamus was sucked out. Again, amplitude decreased. Furthermore, the postcentral pattern became much less regular both in rate and shape of the waves. At times no true rhythm could be made out, at others there were intervals of many seconds in which irregular waves of varying size would appear.

(vi) Finally, by suction, a large part of the hypothalamus was excavated. This was followed by a marked drop in both amplitude and rate and the pattern became less definite and more irregular in both sites. At times a rapid 9-12 per sec wave appeared, at others there were only low and faint oscillations.

The hypothalamic extirpations were made piecemeal in four steps. The change in character of the potentials was gradual and increased following each ablation. The difference between the final record and that with intact hypothalamus was marked. Since there had been some respiratory disturbance associated with these procedures it was thought that a resultant anoxia might have affected the cortex. However, these disturbances of respiration were slight, consisting only of an alteration of rate, or an interruption of respiration for the space of one or two breaths. An EEG made one hour after the final operative procedure showed no recovery of EEG, although the animal was still in excellent condition.

At autopsy the lesions were as follows: the left caudate and almost all the head of the right caudate had been destroyed, about one half of the left putamen was absent, virtually all of both hypothalamus and thalamus had been removed.

**Summary.** Removal of the left hemisphere was followed by the appearance of large, slow waves in right precentral cortex but not in the postcentral region. They were transient. Removal of the left caudate reproduced these waves in right precentral cortex. Ablations of left putamen and right caudate had no further effect on EEGs. But extirpation of the thalamus caused the amplitude to diminish in both cortical areas and further, caused an alteration in the character of the waves, most marked in the postcentral region. Here the rate became slower and the pattern more irregular. Subsequent lesions in the hypothalamus greatly altered the character of the EEG. Amplitude became low and the entire pattern uneven and irregular.

*Expt 5 (EEG 13), Feb 12, 1943.* In this experiment a normal monkey, weighing 5.4 kg, was put under anesthesia and the calvarium removed. The left cerebral cortex was then ablated and bipolar leads placed on right pre- and postcentral cortex. Records were made from these two areas throughout the experiment. A third pair of leads was used to record the EKG in order to observe what effect, if any, lesions of the hypothalamus had on heart rate. Respirations were marked on the record and counted at frequent intervals, for the same reason.

From the right precentral area, following removal of the left hemisphere large rounded slow waves were obtained such as appeared after the same procedure in Expt 4. Again, these waves were present for only about ten minutes. More normal waves then reappeared, which were slightly faster, of lower amplitude and containing both the medium 6-8 per sec rate and a faster component such as is seen in the normal EEG. During this entire period records from the postcentral gyrus had remained unchanged.

The anterior inferior portion of the hypothalamus was then removed by sucker from just above the chiasm. There followed a rather gradual change, taking place over at least 15 minutes after termination of the procedure, during which interval there was a gradual diminution in amplitude of the waves. At the same time the rate slowed and the pattern began to look much less regular.

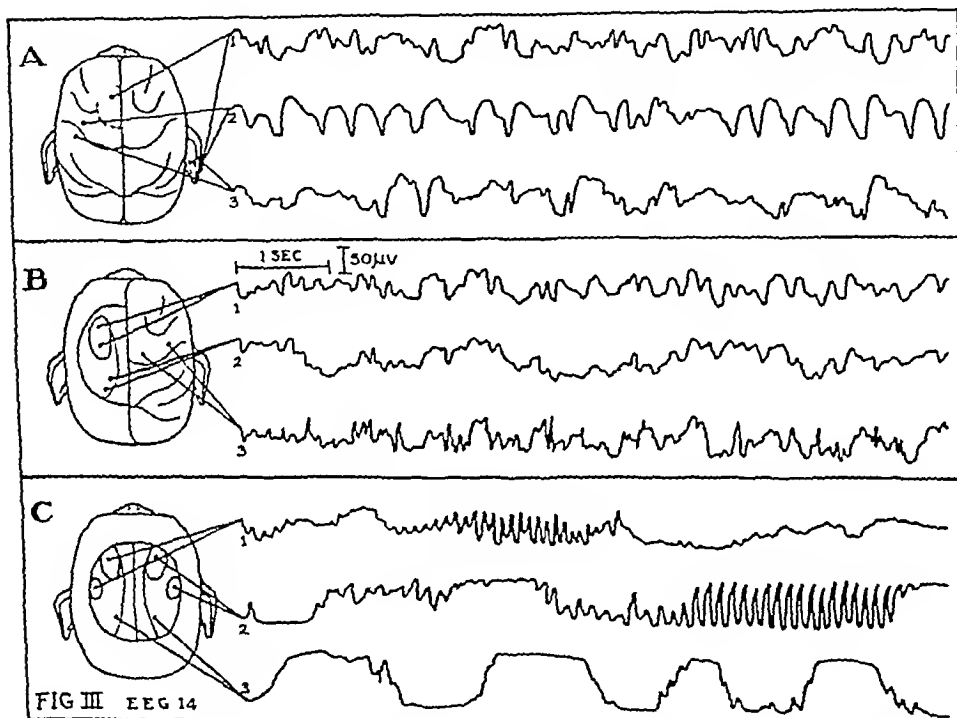
The second procedure was to remove caudate and putamen from the left side. There was no appreciable change in the records which, because of the previous hypothalamic extirpation were definitely irregular and far from normal.

The next procedure was to remove the rest of the hypothalamus from above by entering the foramen of Munro with the sucker and removing all gray matter along the sides of the third ventricle. Respiration and heart rate were unaffected by this. The blood in the cerebral arterioles remained of good color and the pulsation of these vessels indicated a good blood pressure. There followed an intensification of the changes recorded above. Again these appeared immediately after operation and progressively increased over a period of about half an hour. The amplitude during this period diminished greatly and the pattern became

Autopsy findings verified the extent and site of both the lesions and electrodes

**Summary** As in the preceding experiment, hemidecortication, permitted the taking of records from basal ganglia and thalamus. Simultaneous independent rhythms from cortex, caudate and thalamus were obtained under these circumstances, but these became altered when all cortical tissue was removed. The spontaneous bursts of high amplitude spikes were then characteristic of the basal ganglia. Removal of most of the thalamic tissue did not affect these bursts, but ablation of the hypothalamus at once diminished the potentials in all areas until no true rhythmic potentials could be obtained. During the hypothalamus ablation (which followed all other procedures), however, the condition of the animal was not satisfactory.

Since the above three experiments had all shown that the basal ganglia and thalamic



nuclei possessed EEGs which existed after total decortication, but that these patterns were altered by the presence of cortical tissue, the next two experiments were planned for further analysis of the cortico-subcortical interrelations.

**Expt 4 (EEG 15), Jan 20, 1943** A normal monkey, weighing 2.7 kg, was anesthetized and both cerebral hemispheres exposed. Two pairs of bipolar electrodes were then placed on the right cortex, one on pre- and the other on postcentral area and records were obtained from this normal cortex. These leads were then kept in place throughout the remainder of the experiment. The following procedures were carried out:

(i) The left hemisphere was extirpated. There followed a transient disturbance of pattern in the precentral right cortex. It was characterized by large, slow, rounded waves which are indicative of injury. There was no such change in the postcentral area.

(ii) Removal of the left caudate nucleus by suction reproduced these slow waves in the precentral area and they remained there for the duration of the experiment, thus making the character of pre- and postcentral potentials quite different throughout the rest of the procedure.

(iii) Removal of the left putamen by suction caused no further change.

activity from basal ganglia in the literature, there are suggestions of such a phenomenon in two papers Jung and Kornmuller (5), describing a method of recording isolated subcortical potentials, show, in their illustrations, certain irregularities in the records obtained from the basal ganglia which look like these bursts of excitation Gerard, Marshall and Saul (3) using an amplifier from an oscillograph and working on auditory stimuli describe "intense high frequency howls" given by basal regions which "waxed and waned in intensity over a period of a few seconds to three minutes probably as individual units become more or less synchronized "

Individual rhythms were obtained from the thalamus which were alike, whether from midline structures or the lateral parts, from the surface or from the depths of the massa intermedia They differed in rate, amplitude and pattern from simultaneous rhythms recorded from basal ganglia and cortex These thalamic EEGs were of rather low amplitude, although not as low as those from basal ganglia They contained both medium, 8-10 per sec rhythm and faster components They resembled more closely the cortical patterns than those of basal ganglia and, at times but not always, they were synchronous in pattern with the EEG from the postcentral cortex This is in agreement with the findings of Dempsey and Morison (2,9) as is also the observation that injury to thalamus affects cortical areas The present findings fit perfectly into the concept of Dempsey and Morison of the electrical activity of a thalamocortical relay system with a reverberating circuit within which many units are coordinated at several levels This has been analyzed much further by these authors with respect to type of activity and localization within the thalamic nuclei

Both Grinker and Serota (4) and Obrador (10) have reported that the destruction of the hypothalamus in cats abolished the EEG The acute preparations of Obrador showed no return of this function Grinker and Serota report also in acute preparations, that the rhythmic potentials returned after some minutes A similar diminution in amplitude appeared temporarily in the present preparations following partial destruction of the hypothalamus Several small lesions made following each other served to summate so that after the last, there was a much greater deficit than that which followed preceding extirpations The same thing was true with regard to the thalamus, successive partial destruction of thalamic areas was followed at each procedure by diminution of amplitude of EEG and by a loss of definition within its pattern Only when both thalamus and hypothalamus were practically totally destroyed did the EEG disappear and not reappear even after an hour during which the general condition of the animal remained excellent

There is one finding for which there is as yet no explanation, i e , following lesions of both thalamus and hypothalamus there was, for a period of from 15 min to one-half hour after termination of all operative procedures, a progressive diminution of the amplitude and pattern of the EEG from the cortex It is to be inferred that in some way the reverberating circuit through

so irregular that its structure could hardly be determined. There were occasional bursts of rapid pulsations which, unlike those seen from basal ganglia, were of low amplitude and all of one height and rate. The postcentral region was affected before the precentral by this manipulation.

About an hour after this last procedure a large hole was made in the left thalamus. All activity again decreased greatly. Waves became slow, 2-3 per sec, irregular and without pattern. There was no fast component present. Removal of the right thalamus next caused a further decrease in activity so that practically nothing remained except a background wavering of the record which was without true form.

During all this period the condition of the animal was excellent. There was no indication from either respiration or pulse that any change in blood supply was present which could have affected the EEG.

### DISCUSSION

These findings are not wholly new, furthermore, they are what would be expected in the light of present knowledge of the EEG. Nevertheless, from the above five experiments various matters seem to have become clarified.

First, if the normal EEG, as above stated, is the sum of the voltage differences from the entire complex of the central nervous system, then the findings related to purely cortical ablations can be easily explained. The major portion of the entire cellular gray matter lies in the cortex in all primate forms in which cerebral convolutions are more or less complex. This cortical meshwork is directly or indirectly under the influence of all the subcortical nuclei and may therefore be almost homogeneous in respect to its voltage differences. If, then, even relatively large cortical cellular masses are removed it is not surprising that, although local excisions change local responses as in Expts 4 and 5, the general pattern is unchanged, because the normal afferent and efferent relations with subcortical structures are maintained. In contrast, since the relatively small cellular bulk of the subcortical nuclei affects relatively large cortical areas, disturbances of the subcortical complexes must alter the total EEG.

In the above experiments it is adequately shown that the normal potentials obtained from either cortex, basal ganglia or thalamus are the result of voltage differences in all three sites, since the removal of one may alter the complexes elsewhere. Certain areas affect only certain others, however, and have little or no detectable influence on potentials elsewhere. This is shown most spectacularly in the basal ganglia-cortical relations. Lesions of caudate and putamen altered the EEG from the precentral region but not from the postcentral. In the absence of part, or all of one hemisphere, the EEG obtained from caudate or putamen is characteristic for that nuclear complex, but is entirely changed by subsequent total decortication.

The change following decortication cannot be due to temporary effect such as tissue injury or anoxia for, in Expt 1, the left caudate and putamen both gave characteristic background voltages and spontaneous bursts of activity one year after the removal of their overlying hemisphere. It is of passing interest that there is evidence of such function because the basal ganglia have been thought to degenerate in the absence of motor cortex.

Although there is no substantial evidence of the spontaneous bursts of

- 8 KENNARD, MARGARET A , and NIMS, L F    Effect on electroencephalogram of lesions of cerebral cortex and basal ganglia in *Macaca mulatta* *J Neurophysiol* , 1942, 5 335-348
- 9 MORISON, R S , and DEMPSEY, E W    Mechanism of thalamocortical augmentation and repetition *Amer J Physiol* , 1943, 138 297-308
- 10 OBRADOR, S    Effect of hypothalamic lesions on electrical activity of cerebral cortex *J Neurophysiol* , 1943, 6 81-85
- 11 SPIEGEL, E A    Comparative study of the thalamic, cerebral and cerebellar potentials *Amer J Physiol* , 1937, 118 569-579

cortical and subcortical areas takes this measurable period of time to adjust. In contrast, lesions of either cortex or basal ganglia seemed to produce maximal effect immediately at termination of the operation.

There is evidence, from chronic preparations, that similar interdependence between nuclear complexes exists. The effect of lesions of basal ganglia on EEG of chronic monkeys has already been demonstrated, results of experiments concerning thalamus and hypothalamus will be published shortly.

#### SUMMARY

1. Electrical potentials have been recorded from cortex and subcortical nuclei in decorticate and hemidecorticate monkeys.

2. Simultaneous EEGs made from these areas may be independent of one another and the patterns from cortex, basal ganglia, thalamus and hypothalamus are characteristic for each specific area.

3. Each cellular complex, however, influences the other complexes. In particular lesions of the sub-cortical nuclear complexes affect cortical potentials.

4. The EEG obtained from basal ganglia of a hemidecorticate preparation shows low-voltage eight-per-second background potentials. Only when all of the cerebral cortex is removed do spontaneous bursts of high-voltage activity appear.

5. The pattern obtained from thalamus has a medium-rate component, as in the cortex. At times records of thalamus and postcentral cortex are synchronous. Lesions of thalamus alter the pattern of cortical EEGs in general but most markedly in the postcentral areas.

6. There is little activity within the normal hypothalamus but its ablation profoundly alters cortical EEGs.

8. Partial ablation of thalamus or hypothalamus temporarily alters cortical EEGs, but only when both thalamus and hypothalamus are completely extirpated are these cortical potentials abolished.

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instrument Photographic negatives were then made of a suitable section and of the mount of the records, the sketch of the section on the latter having been erased Suitable magnification of the section and reduction of the mount were made such that they could be superimposed to give a final print with a magnification of the section at  $10\times$  and a reduction of the record mount to one-third When using material stained with the Weil method, a negative of the section was masked with a positive of the mount The final print thus contained a positive of the section and a negative of the electrical activity (cf Fig 4, 6, and 8) With Nissl material superposition of two positive prints was made by the method of double exposure (Fig 5 and 7)

Accurate register was obtained in both instances by reference to horizontal and vertical lines scratched on the original slide at some distance from the field of interest and included in the tracing used for the record mount

## RESULTS

*Thalamus* In spite of the great difference in conditions encountered deep within the thalamus from those in the cortex, records from the two organs showed strikingly similar features The intermittent "burst" activity, re-

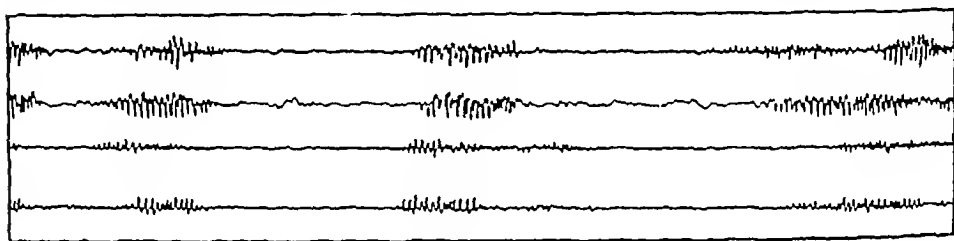


FIG 1 Synchrony of burst activity in thalamus and various parts of cortex Record begins just before the end of a burst Upper tracing from point in thalamus similar to that for tracing 6 (from top down) in track 1 (most medial) Fig 5 (but not the same experiment) Second tracing anterior sigmoid gyrus, third tracing arm sensory area, lowest tracing middle suprasylvian gyrus The record was taken at a paper speed of 15 mm per sec and reduced to  $\frac{1}{3}$

ferred to above as characteristic of the cortex of cats under nembutal, occurred in similar form in specific parts of the thalamus In many cases the frequency of occurrence of the bursts and that of the waves within them, though of similar general character, were not identical with those emanating from any particular cortical position In others striking similarity with one

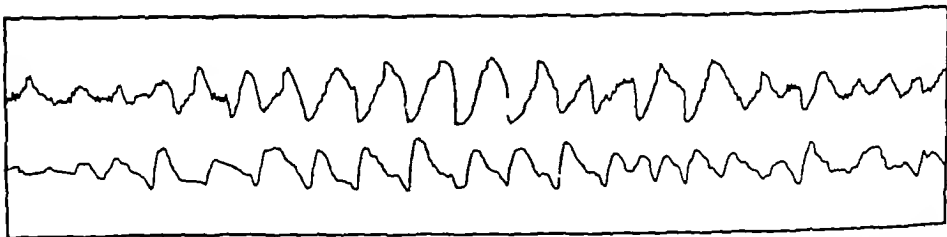


FIG 2 Approximate wave to wave correspondence between thalamus and cortex Upper tracing same as tracing 6, track 1, Fig 5 Lower tracing anterior sigmoid gyrus Paper speed 60 mm per sec (reduced to  $\frac{1}{3}$ )

# SPONTANEOUS ELECTRICAL ACTIVITY OF THE THALAMUS AND OTHER FOREBRAIN STRUCTURES

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STIMULATION of thalamic regions associated with the internal medullary lamina has given rise to alterations in the electrocorticogram which appear to be related to the spontaneous intermittent bursts of 5-10 cycle activity characteristic of the nembutalized cat (11). On the other hand, stimulations more laterally in *N. ventralis lateralis pars externa* (6) or *ventralis posterior* of Magoun and McKinley (8) and the geniculate bodies produce changes which can be shown to bear relation to other more continuous forms of cortical activity (1) in localized cortical regions receiving their projections.

The present study was undertaken in order to find out whether these two different thalamic regions exhibited characteristic differences in their spontaneous activity.

Though specifically directed to the preceding question, the experiments also yielded data regarding the activity of other structures which, though limited in scope, appear to be worth reporting in view of the incomplete nature of existing information.

The most complete surveys of the spontaneous electrical activity of the basal prosencephalon are those of Gerard, Marshall and Saul (3) and Morea (10), but the general utility of the former is somewhat limited by the fact that the records were made largely in the form of verbal descriptions of the sounds emanating from a loudspeaker. In the present study, the admittedly greater differentiation allowed by their procedure has been sacrificed for the greater permanence and transmittability of the graphic method.

## METHODS

Cats anesthetized with nembutal were used, an effort being made to adjust the anesthesia to a point at which well defined intermittent bursts of 5-10 cycles were recorded from the cortex. The exploring electrodes consisted of two enameled stainless steel wires separated at their bared tips by a distance of 0.3 to 6.0 mm. These were oriented in the brain by means of the stereotactic instrument previously described (12). In most of the experiments other bipolar silver electrodes were placed on representative cortical areas in order to obtain data for correlation with activity in deeper structures. In other experiments various cortical ablations were made. The recording instrument consisted of a multichannel Grass inkwriter.

At the termination of each experiment the brain was removed and fixed in formalin for sectioning and staining by the method of Marshall (9). Direct positive enlargements were made from the sections and the points of recording identified on the prints.

In order to obtain a graphic representation of the distribution which would appeal more immediately to the eye, the following method was employed.

Representative cross sections were projected at 30 times magnification and sketches of important landmarks and the needle tracks were made. Typical excerpts of the records were then mounted upon the sketch with reference to the coordinates of the stereotactic



FIG 4

FIG 4-8 Distribution of spontaneous electrical activity of thalamus and other basal areas in a single representative experiment For technique of constructing figures consult *methods* The electrical records were taken at 15 mm per sec and reduced to  $\frac{1}{2}$  The brain sections were enlarged  $10\times$

Typical bursts were selected from areas showing intermittent activity All other records are representative of the continuous activity present The vertical markers to the left of some tracings in track 1, Fig 5, indicate  $50\mu\text{V}$  All records in Figure 4 and the first three in Fig 5 were taken with amplification as in tracing 3, all subsequent to tracing 6 as in tracing 6

There was a slight displacement of the needle tracks from the plane of section in some instances This was so slight as to be negligible except for a few points deep in the amygdala and pyriform lobe in which the records may have been taken from points as much as  $4\text{--}500\mu$  rostral to the points represented

or more of the surface positions was obtained (Fig 1) In only a few instances, however, was a one to one correlation of individual waves possible, the most striking case occurring in a preparation rather deeply anesthetized and showing practically no interburst activity (Fig 2)

Intermittent bursts of this sort were recorded from a rather wide area in the thalamus, especially in medial and intralaminar regions Figures 4-8 illustrate the distribution in a typical experiment In most of the experiments some bursts were recorded laterally and dorsally in the thalamus (cf Fig 5, trace 1 and 2, track 3, and Fig 6, trace 1, track 2 and 3, also the dorsal

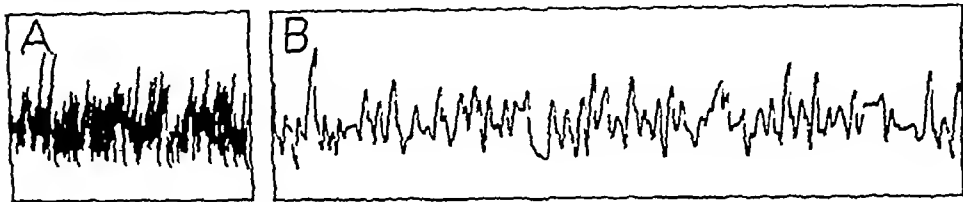


FIG 3 Spontaneous activity of point similar to 6th tracing, track 5, Fig 6 At two different paper speeds A, 15 mm per sec, B, 60 mm per sec

area in Fig 7 which was not explored in the case illustrated and may be attributed to lateralis posterior or pulvinar) In most of these, however, the highest voltage and greatest frequency of occurrence were found more medially Coordination with the cortical activity was also greater in the medial areas Burst activity has never been encountered in pars externa or the medial and lateral geniculate bodies Fortunately one does not have to rely wholly upon histological checks for the determination of the recording point in these relay nuclei, since they are the seat of distinctive potentials evoked by stimulation of the appropriate sensory receptor (8) As Dempsey and Morison (1, Fig 5) have shown, burst potentials did not occur in areas where sensory responses to radial or sciatic nerve stimulation were recorded In the present series of experiments this point was repeatedly checked and extended to include areas responsive to stimulation by light or sound

Occasionally bursts appeared in the vicinity of ventralis medialis (cf Fig 6, trace 4, track 2) which might be attributed therefore to a relay nucleus since this area has been shown (8) to receive the trigeminal lemniscus Since this would be the only relay nucleus exhibiting such activity in these experimental conditions, it seems more desirable to attribute the activity to the immediately adjacent centralis lateralis or center median

In some experiments, ventralis lateralis appeared to be the site of some bursts, in others they were absent though present in the more medial areas The interpretation of this finding is obscure

In preparations which exhibited little or no burst activity in the cortex, correspondingly poor results were obtained from the thalamus Particularly instructive were cases in which the activity disappeared from the cortex, having once been present or, conversely, developed after having been absent,



FIG 6

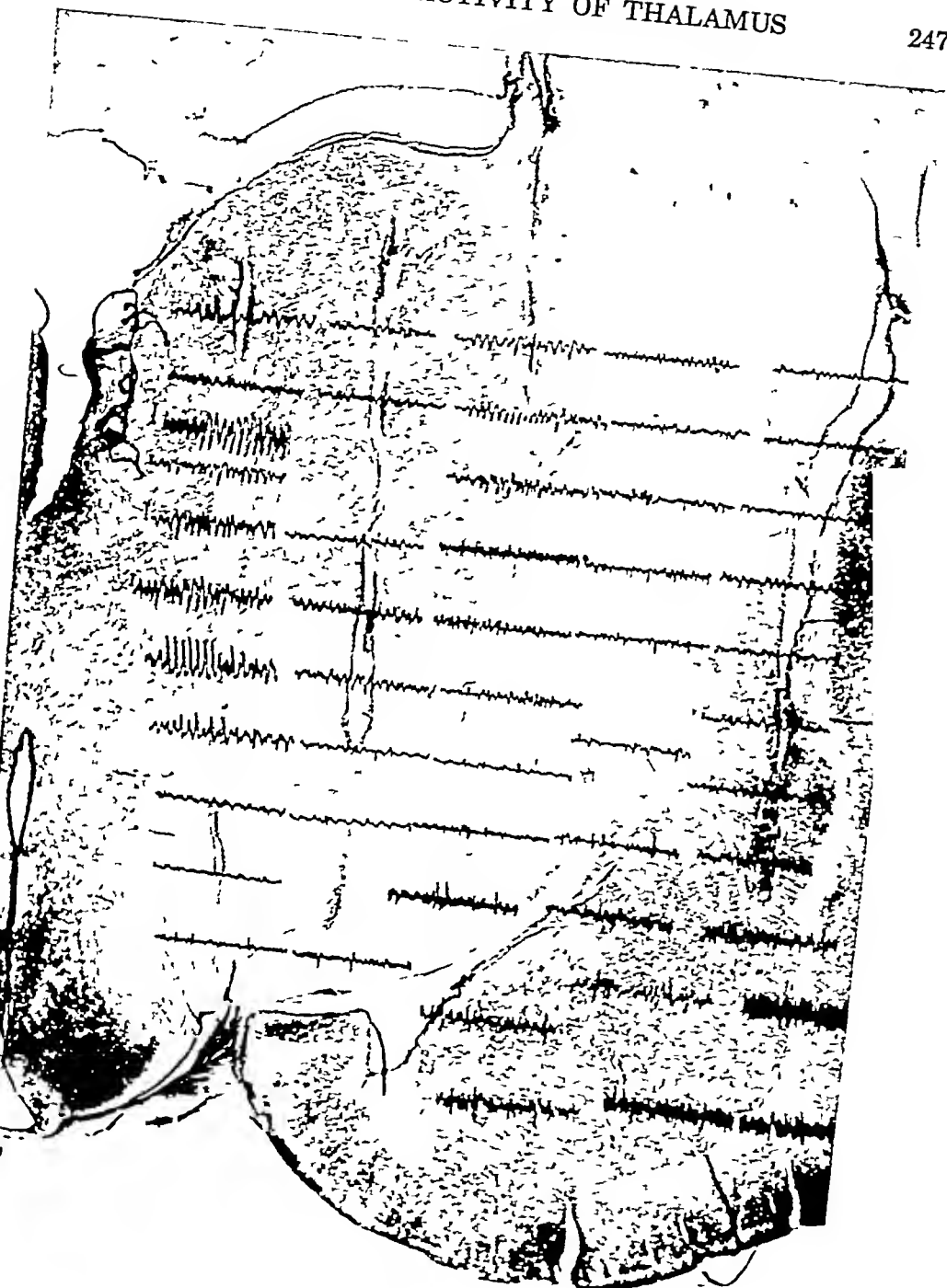


FIG 5



FIG 6

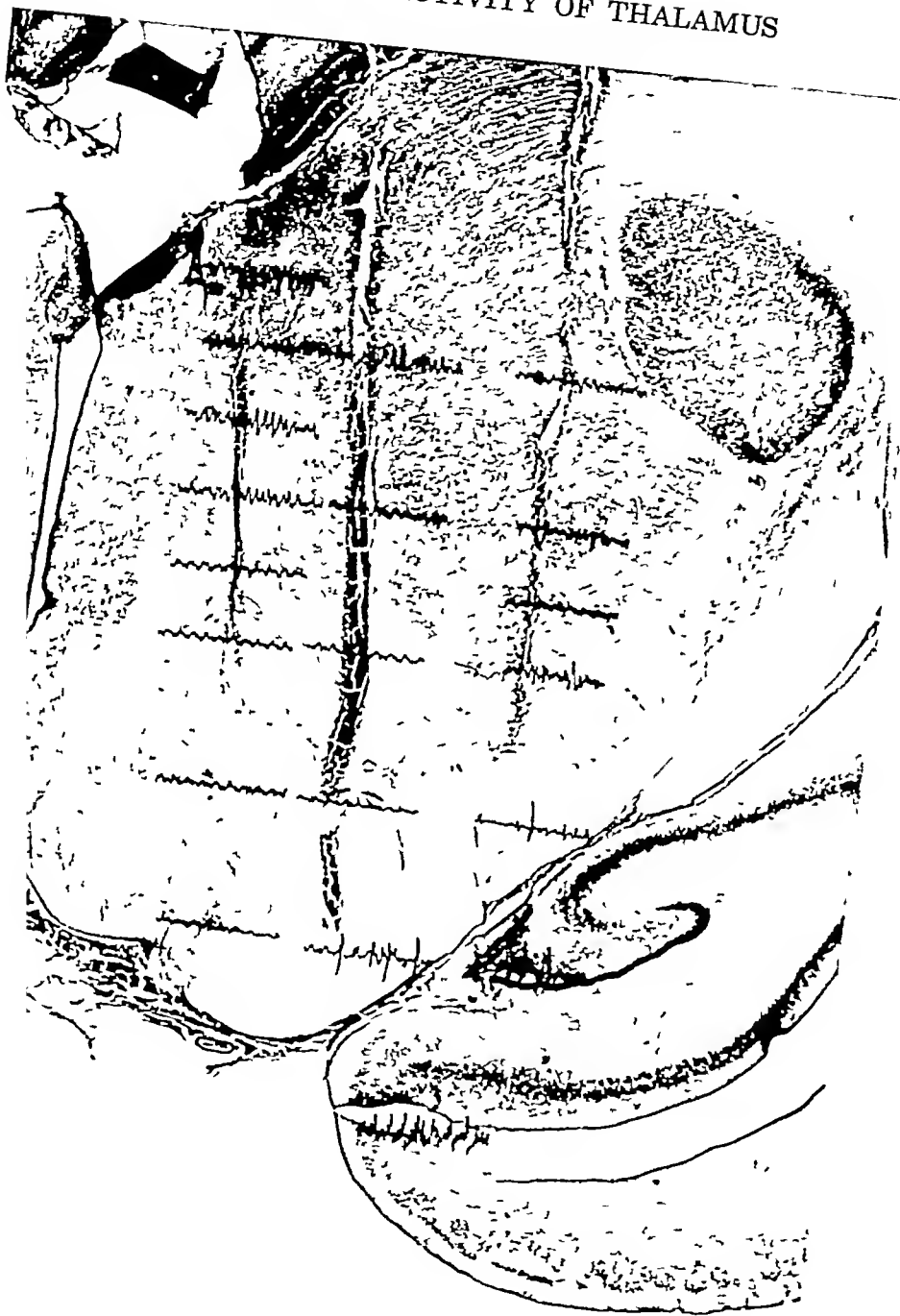


FIG 7



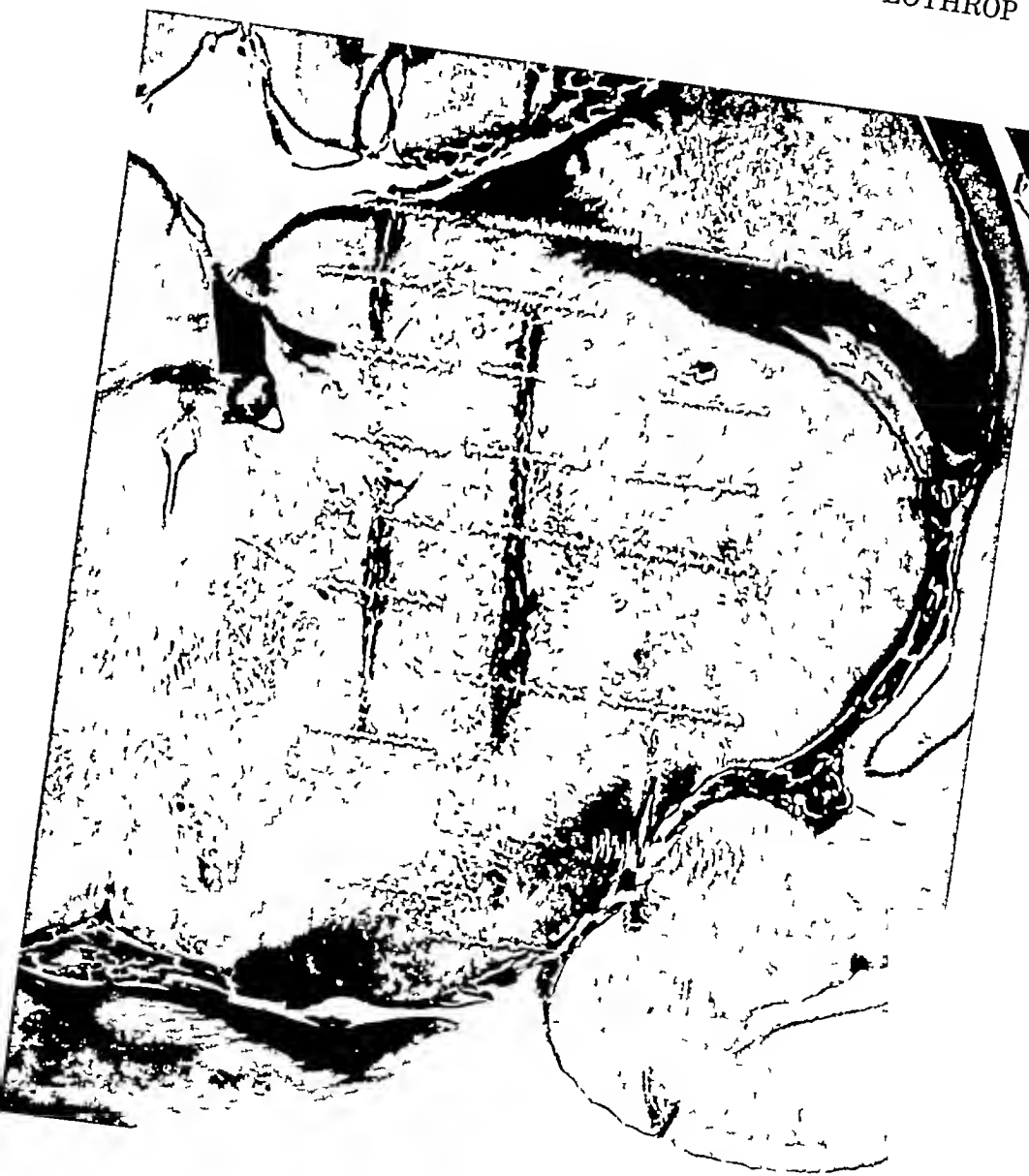


FIG 8

the changes in the thalamus paralleled those in the cortex. On the other hand, excellent bursts have been recorded in the thalamus of preparations with large homolateral cortical defects. In one instance in which all the neopallium of one side was removed, a complete map of the burst activity in the thalamus was made which coincided closely with that in intact animals.

In another case, besides unilateral removal of the cortex, an incision was made separating the two hemispheres as far back as the mammillary bodies. Celloidin sections revealed that a few fibers of the supramammillary decussation and about one-half of the posterior commissure were spared. Good bursts were also recorded from this preparation for a period of about one-half hour.

On the basis of such experiments it is difficult to say whether the spontaneous bursts are a property solely of the thalamus or depend upon the presence of the cortex. Either the early presence of activity after the extensive operative procedure or its subsequent decline might be due to unspecific traumatic factors. Further work in chronic preparations may serve to resolve such questions.

Activity in the relay nuclei (pars externa and geniculates) in these experiments was usually confined to continuous rapid fine spikes of somewhat higher voltage than in surrounding areas (cf. tracing 4 and 5 in track 3, Fig. 6, etc.). Occasionally, when the anesthesia was light, there emerged more organized patterns very similar to those recorded in sensory cortex under proper conditions (cf. Fig. 11 in the paper by Dempsey and Morison, 1). Decortication sharply reduced activity in these nuclei (2) in contradistinction to its apparent lack of effect on the bursts recorded elsewhere.

*Hippocampus fornix system.* High voltage spikes of fairly rapid frequency were usually encountered when the electrodes penetrated the hippocampus or fornix dorsal to the thalamus and tended to disappear over a period of several minutes. No specific attention was directed to them as this system has been studied intensively by others (13). It may, however, be worth recording that in one decorticate preparation two spontaneous "tonic clonic" episodes entirely similar to that illustrated by Rosenblueth and Cannon (14) as the result of faradic stimulation were observed.

*Hypothalamus.* Records taken from the posterior hypothalamus and tuber region consisted almost solely of fine irregular rapid potentials without notable peculiarities. The supraoptic region, however, sometimes yielded more definite waves of 10-12 per sec. frequency. These types of activity were similar to those pictured in Fig. 2 of the paper by Hoagland and others (5). As the latter workers point out, the nuclear complexity of the supraoptic region and its environs necessitates caution in assigning a definite source to the potentials encountered. Under the present experimental conditions, moreover, nothing was found to suggest an intimate relation between cortical activity and that of the hypothalamus or of any part of the supraoptic region (4).

*Other areas.* Spikes of slow and often irregular rhythms upon nondescript more rapid low voltage background were occasionally recorded from lateral

subthalamie areas and in the neighborhood of the globus pallidus, peduncle or possibly the substantia nigra

Most dramatic results were obtained when the electrodes left the diencephalon and penetrated telencephalic structures beneath or laterally. Owing to the semidetached nature of this part of the brain, which includes the pyriform lobe, amygdala and hippocampus, identification of the exact recording point is difficult, especially in regard to the dorsoventral coordinates. Higher voltage rapid activity (Fig 3) was always found widely scattered throughout this region both posteriorly where the hippocampus might be responsible and also more rostrally (Fig 4 and 5) where the amygdala or pyriform lobes were more likely sources. The intensity of this activity declined slowly as the electrodes remained in one position, but was still marked after periods as long as 20 minutes. It thus became a matter of opinion whether the decline was a matter of recovery from injury or, conversely, was due to accumulations of shunting fluid about the electrode tips.

Similar but usually less striking effects were found when the electrodes were in the ventral parts of the putamen or globus pallidus (Fig 4 and 5). This may, however, merely represent spread from amygdala.

No statement as to the activity of the caudate nucleus is to be made on the basis of these experiments as the proximity of the ventricle makes depth determination of the electrodes very difficult with the technique employed, i.e., the large size of the ventricle at this point may be considerably varied by shifts in spinal fluid, or other temporary distortions may be brought about by the penetrating electrodes without the change being registered on the final section.

## DISCUSSION

Principal interest in this study attaches to the distribution of the spontaneous burst activity in the thalamus. This was found to be most intense in regions dorsal and especially medial to the relay nuclei, an arrangement which is consistent with other data (1) which suggest that the latter structures are not primarily related to the intermittent bursts recorded in the cortex. The distribution coincides rather closely, however, with points stimulation of which was followed by an increase in the bursts throughout the neocortex (11).

The active points were dispersed beyond the limits of any single thalamic nucleus but most intense activity was usually found in the intralaminar group. Activity encountered in dorsolateral regions containing nuclei related to "association areas," is in general consistent with the previous finding that association areas exhibit burst activity most intensely (11). Whether or not this activity originates purely in the intralaminar nuclei, such as the centre median or centralis lateralis, and involves more lateral and dorsal regions secondarily is difficult to determine. Such primacy of the intralaminar group is suggested by the usually greater ease of eliciting the "recruiting response" by stimulating these areas, and by the fact that the recorded activity was usually greatest here.

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giving rise to "non-specific cortical afferents" have a tendency to produce spontaneous rhythmic bursts, but that they may be under the additional control of a master area associated especially with the internal medullary lamina

2 Characteristic spontaneous activity in other subcortical regions was recorded and is briefly noted

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As is well known, the bursts, though tending to occur simultaneously in all cortical areas, by no means always do so. Similarly the present results allow the deduction that the thalamic bursts are not simultaneous in different parts of the thalamus. For example, in the same experiment a given thalamic area might be simultaneous with say the cortical sensory area. A change of a few millimeters in electrode position might result in bursts bearing no relation to the sensory area but, for instance, to the middle suprasylvian gyrus. Frequently no relation to any of the cortical areas concurrently recorded could be made out. The finding of different rhythmic patterns in different parts of the thalamus, especially differences in frequency of incidence of the bursts, though difficult to render in quantitative terms, also supports the idea that specific parts of the thalamus are at least semi-independent in the production of burst activity.

The finding of excellent burst activity in the thalamus deprived of all homolateral neocortical connections and a high proportion of interhemispheric commissural systems is of particular interest. As was pointed out above, these experiments are not conclusive, but they suggest very strongly that such activity does not depend upon the presence of a reverberating corticothalamic circuit. Activity in relay nuclei, on the other hand, is usually severely depressed, though not abolished, by interference with cortical connections. Though further work is necessary to establish the hypothesis, it may be permissible to speculate upon the arrangements underlying the spontaneous bursts. One area in the neighborhood of the centre median may be thought of as having the greatest tendency to produce intermittent burst activity. It also is presumed to have rich connections with other more widely diffused thalamic cell groups associated with but not identical to the nuclei giving rise to "specific" cortical afferents. The latter nuclei have been described by Lorente de Nó (7) as giving rise to "non-specific afferents" of a diffuse sort which fit well the requirements of the system under discussion. These nuclei may be postulated to have a tendency toward spontaneous activity of their own and so account for the lack of synchrony often encountered in divers cortical and thalamic areas. On the other hand, they may under other conditions be relatively easily controlled by the "master" oscillator in or near the center median. The latter supposition accounts for the cases of spontaneous synchrony frequently encountered and especially for the fact that stimulation of this area is most effective in eliciting the "recruiting response" (11) simultaneously throughout the cortex.

#### SUMMARY

1 The spontaneous activity of subcortical forebrain areas was recorded by means of bipolar electrodes oriented with a stereotactic instrument. Spontaneous bursts of 5-10 per second waves similar to those seen in the cortex were recorded in various thalamic areas (Fig 4-8), chiefly in those associated with the internal medullary lamina but never in the "relay nuclei." These and other data suggest that several thalamic areas presumably

# HEAD INJURY

## GUN SHOT & PENETRATING WOUNDS

### PENETRATION COMPLETE & INCOMPLETE WITH VARYING VELOCITY



















PHYSIOLOGICAL EFFECT				
PROFOUND (ANIMAL WILL DIE)			  	  
MODERATE (ANIMAL MAY LIVE OR DIE)		 	  	
MINIMAL (ANIMAL WILL LIVE)	 	  	 	
	MECHANICAL DRILL	NON PENETRATING AND TANGENTIAL WOUNDS	22 BB REVOLVER 780 FT SEC (RIFLE)	22 SHORT RIFLE 970 FT SEC

FIG 1 Showing the distribution of physiological effect in relation to the sustained injury. The bullet of the 22 BB revolver weighed 20 grains and that of the 22 short rifle, 40 grains.

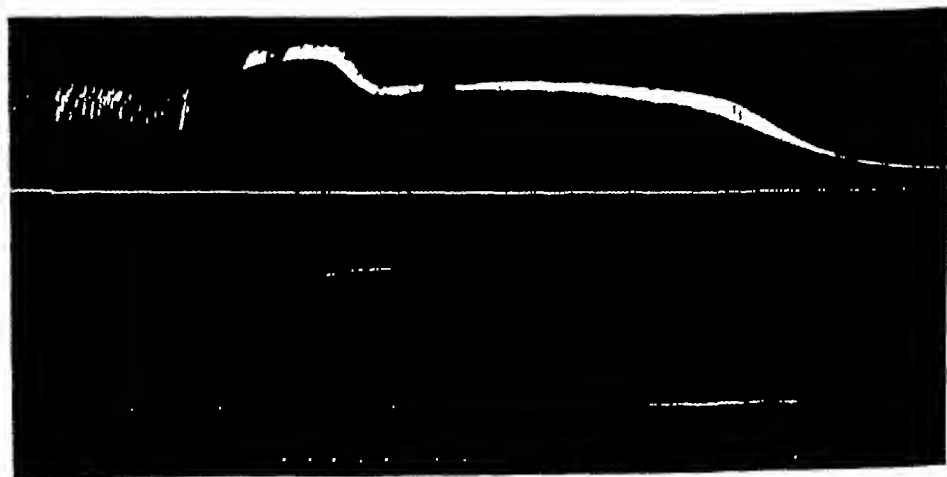


FIG 2 Morphine analgesia. Typical curve following a 22 short rifle injury. The bullet entered the anterior fossa of the skull in front of the sella. Profound effect was produced. There were petechial hemorrhages in the pons.

# ACUTE PHYSIOLOGICAL EFFECTS OF GUNSHOT AND OTHER PENETRATING WOUNDS OF THE BRAIN

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IN THE STUDY of experimental cerebral injuries in the dog, the acute physiological effects of gunshot and other penetrating wounds appeared to be of particular interest. These observations are, therefore, reported in detail.

## TECHNIQUE

Mongrel dogs weighing between 7 and 15 kg were used for this study. The experiments were performed under morphine analgesia in quantities of 20 mg per kg. The blood pressure was recorded from the femoral artery, respirations, by means of a balloon about the thorax. The spinal fluid pressure was recorded from a needle inserted in the cisterna magna and attached to a 1 mm bore water-manometer. Six-tenths cc of cerebrospinal fluid was required to fill the system to starting pressure. Difficulties were encountered in recording the intracranial pressure at the instant of a bullet's penetration. Various systems were employed. Only a qualitative change in pressure could be recorded. Bullets of varying velocity and size produced the injuries. These were the pellet, the bullet of the 22 BB revolver which weighed 20 grains and had a speed of 780 ft per sec and the bullet of the 22 short rifle which weighed 40 grains and had a speed of 970 ft per sec. In a total of 24 animals, 3 were injured with pellets from an air revolver, 16 with a 22 BB revolver and 5 with a 22 short rifle. A mechanical drill was used to obtain a penetrating injury of minimal velocity and this was done in two instances. As a rule, the animals were injured with the soft tissues intact and the cranial cavity closed. In a number of instances, the wounds were made after disturbance of the closed cavity hydrodynamics by opening the cistern to expose the medulla, suboccipital craniectomy to expose the cerebellum and brain stem and unilateral or bilateral supratentorial craniectomy to expose one or both hemispheres. Infiltration of 1 per cent procaine hydrochloride was used in these procedures for local anesthesia.

## RESULTS

1 *Effects upon blood pressure, respirations, reflexes, conscious state and mortality.* The results of injuries produced by the pellet shot, the 22 BB (revolver), the 22 short (rifle) and penetration of the brain by a mechanical drill could be classified into *profound*, *moderate* and *minimal* physiologic effects as shown in Fig 1.

*Profound.* A profound result was produced by 22 rifle injury in every instance. Among 7 of 14 experiments with 22 revolver injury, profound effects were also produced. No such effects were noted with the pellet shot. The profound effect was uniformly characterized by respiratory paralysis, abolition of lid and corneal reflexes, a marked hypertension and death of the animal (Fig 2). Coma\* was immediate. Extensor spasms were absent in most instances. The hypertension did not occur in those experiments in which the medulla was directly injured (Fig 3). It also failed to appear after adequate

\* Coma. A state of absolute unconsciousness as judged by the absence of any psychologically understandable response to external stimuli or inner need. (British Research Council, Head Injury Committee, 1941)



decompression before injury by shooting (Fig 4) In the latter group, the respiratory function was spared in the acute phase of the experiment

The pathological damage, under these circumstances, was of severe grade A common finding was the presence of petechial hemorrhages These were of varying size and number, occurring adjacent to the site of penetration, the pons and frequently in the medulla and upper cervical spinal cord

*Moderate* A moderate effect resulted from injuries produced by the 22 BB revolver in 7 instances out of 14 The animals in this group either died or survived the acute experiment Temporary arrest of respirations occurred for periods which varied from five to thirty seconds Lost palpebral reflexes

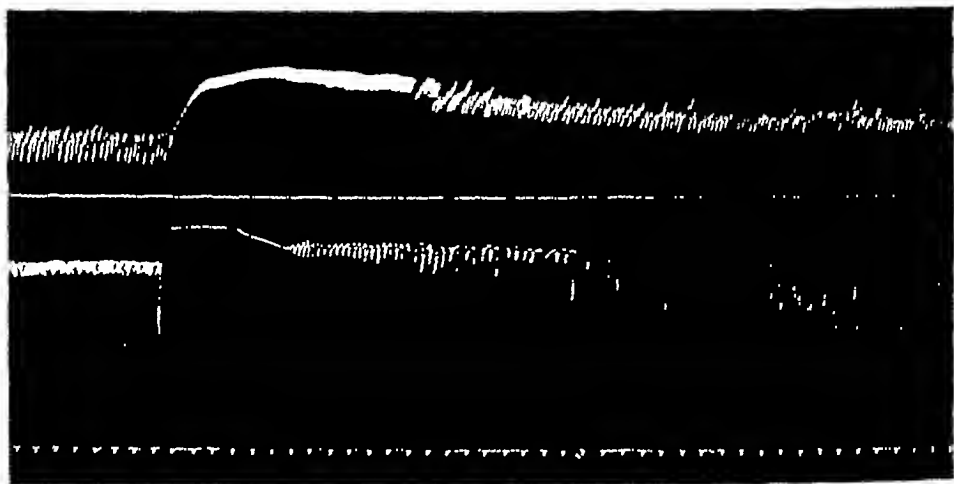


FIG 5 Morphine analgesia Injured with a 22 revolver, bullet lodged in the brain. Cessation of respirations for 35 seconds but reflexes were present throughout The dog was unconscious The lid reflexes may not serve as a criterion for accurately determining consciousness The spinal fluid was bloody with a pressure of 115 mms Type of injury, moderate

returned at periods of from 3 to 145 seconds A rise in blood pressure with tachycardia was consistently observed (Fig 5) In some experiments there was a dissociation of conscious state and the palpebral reflexes, that is, some unconscious animals had preserved reflexes

The gross pathologic damage was less severe than in the preceding group The bullet usually lodged in the cerebral substance Distant lesions were less frequent

*Minimal* A minimal effect followed trauma by the pellet shot in 3 instances, by the 22 revolver in 2 instances out of a total of 14, and by the use of the hand drill With gunshot injuries producing a minimal effect, there was fracture of bone without penetration or the bullet stopped at the skull without injury to bone By the use of a mechanical drill, both bone and brain were penetrated Members of this group all survived the experiments Respirations and reflexes were preserved In some animals the respirations were

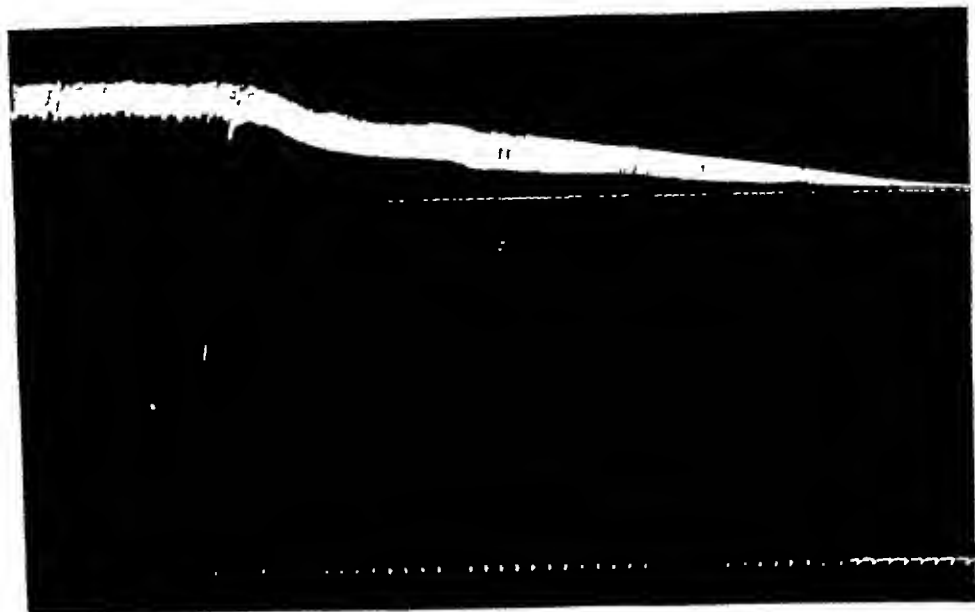


FIG 3 Morphine analgesia 22 short rifle injury, the bullet entering the medulla A profound effect was produced without elevation of the blood pressure

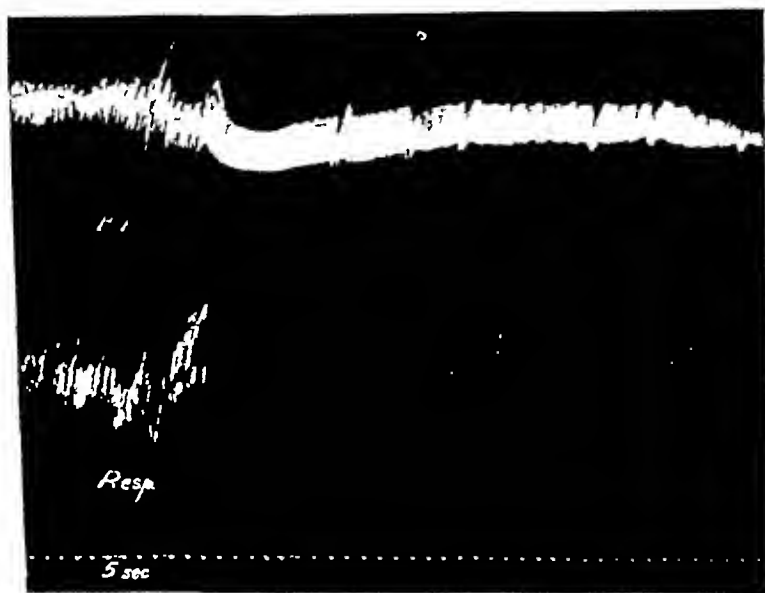


FIG 4 Morphine analgesia 22 short rifle injury following bilateral decompression exposing both cerebral hemispheres No rise in blood pressure Reflexes lost for 2 minutes and 30 seconds Respirations lost for 40 seconds The animal remained unconscious throughout With the cranial cavity closed, this type of injury would be fatal with a pattern as illustrated in Figure 2

This animal survived an injury which would be fatal under the condition of a closed cranial cavity. In this instance, the increase in intracranial pressure was prevented by the decompression.

### DISCUSSION

In the above described experiments, a mechanical drill run by hand was used to penetrate the brain and three types of bullets were used for the gun-

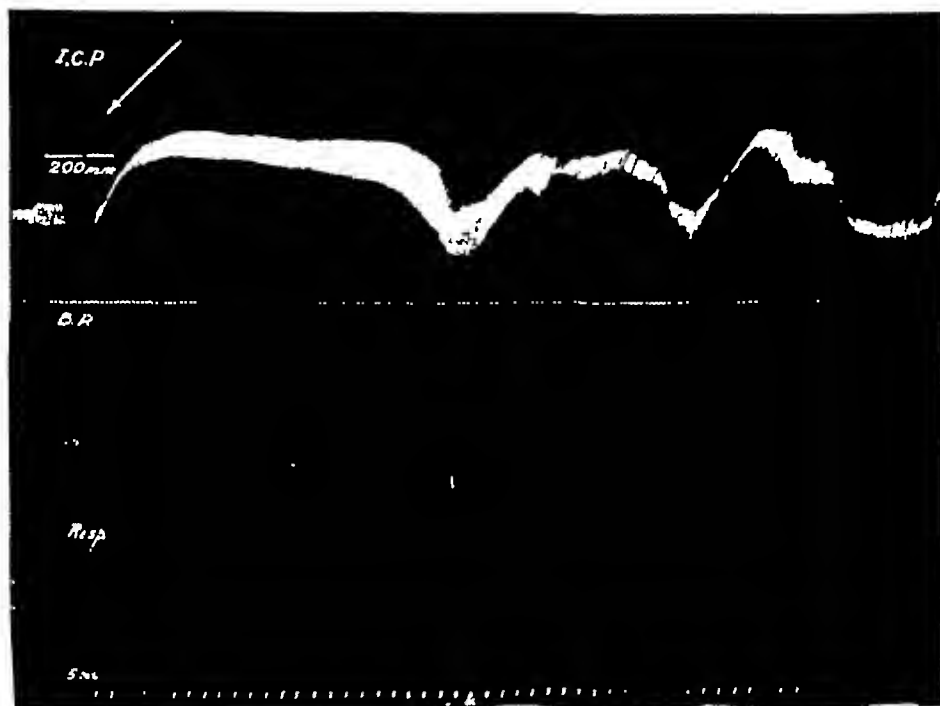


FIG 7 Morphine analgesia. Injured with 22 revolver. The intracranial pressure recorded a sharp qualitative change at the instant of injury (see arrow). Respirations ceased and reflexes were lost temporarily. With return of respirations, there was a corresponding rise of the blood pressure suggesting that in this instance, the vasomotor mechanism was failing before the respiratory function.

shot injuries—the pellet (revolver), the 22 BB (revolver 780 ft per sec) and the 22 short (rifle 970 ft per sec). It is evident from figure one that the greater the velocity the greater was the severity of the pathological and physiological effects. Whereas with the 22 short rifle (970 ft per sec) only profound effects were produced, with a pellet injury, no profound effects were obtained. It should be stated that the weight of a bullet is also significant. The 22 BB shot weighs only 20 grains whereas the bullets used for the 22 short rifle were 40 grains. Therefore, the results from a gunshot injury must be ascribed not only to the velocity but also to the mass of the bullet. A relatively great velocity of an extremely small mass may produce minimal

stimulated, in others, slowed. Consciousness was maintained. The blood pressure usually remained unchanged (Fig 6). Pathological examination of the specimens from this group indicated mainly local damage with or without hemorrhage from the site of injury.

2 *Effect upon the intracranial pressure* A sudden, intense, increased intracranial tension of brief duration was observed to accompany the gunshot injuries of the brain. Evidence of an intense pressure was visualized by exposure of the cisterna magna. Under this condition, at the moment of injury, the cerebellum was expelled into the opening made. A suboccipital craniectomy followed by a supratentorial, gunshot injury was observed to result in

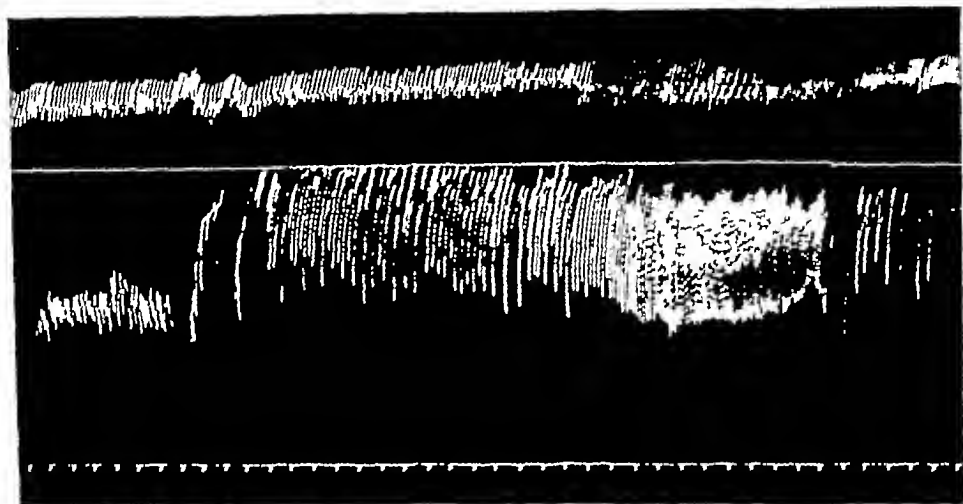


FIG 6 Morphine analgesia. Penetration of frontal lobes with a hand drill. Reflexes present. Respirations were stimulated. Blood pressure remained about the same throughout. The animal was conscious. Spinal fluid slightly bloody with a pressure of 400 mms. Minimal effect.

expulsion of the cerebellum with such force as to lacerate that tissue. An injury with a 22 BB rifle 5 cm below the medulla which transected the spinal cord, resulted in cerebral contusions of the cortex of the hemispheres due possibly to transmitted pressure. Finally, cerebral tissue was consistently extruded through the defect made by a bullet's entrance.

Measurement of the intracranial pressure at the time of injury by means of a fluid system attached by copper tubing to a tambour from an adapter screwed into the skull, showed only instant qualitative changes in pressure. Records of the cerebrospinal fluid pressure in the cistern at later intervals by means of a 1 mm bore manometer showed the highest pressure to be 400 mm of water. The average pressure was between 100 mm and 200 mm of water. Figure 7 is a typical record of blood pressure and the respirations, showing also the qualitative change in intracranial pressure at the moment of injury. Figure 4 shows the result after decompression of both hemispheres.

ing object The increased pressure is dispersed throughout the closed system, it acts upon all tissue and it is manifested by the response of the vital medullary centers The vital cell or center damage, recoverable or fatal, may be the direct result of this sudden pressure Additional immediate or delayed injury to the cells may result from the indirect effects of intrinsic vascular damage and the local destructive process When the medulla was destroyed, death occurred without hypertension because of dissolution of the vasomotor mechanism By means of a bilateral decompression, the intracranial hypertension was partly prevented and the medulla centers were spared

### CONCLUSIONS

1 Penetrating wounds of the brain were produced in dogs under morphine analgesia by means of a mechanical drill, pellet shot, 22 BB revolver and a 22 BB rifle

2 A sudden, intense, increased intracranial tension of brief duration at the instant of injury was observed to accompany gunshot injuries of the brain by the 22 BB revolver and BB rifle It is felt that this increased intracranial tension is the cause of the acute physiologic effects observed

3 The results of the injuries could be classified into profound, moderate and minimal physiologic effects Profound effects were characterized by loss of respiratory and palpebral reflex activity, hypertension and death, the result of injury with the 22 short rifle (970 ft per sec ) In the moderate group, the reflexes and respiratory function were temporarily interrupted, a rise in blood pressure usually occurred, some of the animals survived This degree of injury was produced by the 22 BB revolver (780 ft per sec ) Those in the minimal group showed no significant change in the vital functions and these animals all survived the acute experiment in which injury was produced by a penetrating drill or pellet shot

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injury while a large mass with minimal velocity may produce no significant effect. The product of mass and velocity is momentum. In our experience, the acute pathological and physiological effects of injury to the head are proportional to the time rate of change of momentum of the injuring object within an optimum range.

Sir Victor Horsley, (1) observed the effects of gunshot wounds of the brain in the experimental animal. He emphasized the significance of a bullet's rotation as it penetrated, the rotation setting up perpendicular lines of force and thus producing widespread damage. Kramer and Horsley (2) stated that death "is not, as usually supposed, due to failure of the heart, but to arrest of the respiratory movements." The changes in circulation consisted of "(a) slight initial fall of blood pressure, (b) considerable later rise of blood pressure, (c) preservation of the rhythm of the heart." The authors attributed these phenomena as due to "a gradual rise in the intracranial tension due to hemorrhage within the skull cavity"—"supervening on the primary arrest of respiration." They stated that "the action of a bullet is essentially a hydrodynamic one."

The observations which have been made are similar to those of Kramer and Horsley concerning the vulnerability of the respiratory center in gunshot wounds of the brain. The response of the blood pressure to this type of trauma was different from that reported by these observers. In our experience, a rise in blood pressure occurred which was prompt, profound and uniform in the higher velocity group of injuries. The hypertension began at the instant of injury and it was not preceded by a fall in pressure. Tachycardia and an increased pulse pressure usually accompanied the hypertension.

The lid and corneal reflexes, the respirations and vasomotor activity were deranged in direct proportion to the intensity of brain stem injury. In the profound group, the reflexes and the respirations were abolished. In the minimal group, although minor disturbances of respiratory rate occurred, reflex function appeared to be normal. Unpredictable reactions of the respirations and reflexes were noted in the moderate group. In some experiments, respiratory activity alone was lost while the lid reflexes remained active. In others reflexes only were lost. In most instances, both respiratory and reflex function were lost together and each recovered at varying and different periods. The absence of lid and corneal reflexes was always associated with coma. However, coma was also found to occur in the presence of active lid and corneal reflexes. Thus, the absence or presence of these reflexes, a criterion used by Denny-Brown and Russell (3) may not serve for evaluating the conscious state.

The acute physiological response following gunshot penetrating wounds of the brain in the experimental animal is due to an intense, momentary increase in intracranial tension at the instant of injury. The force, (known as "explosive force") (4), of a bullet transmitted to a closed, semifluid system, results in a sudden increased intracranial pressure, the intensity of which is dependent upon the time rate of change of momentum of the injur-

motility in the proximal segment of the transected intestine following distension of the distal segment could still be obtained in some animals after division of the nerves indicated above. The intestino-intestinal inhibitory reflex, however, could only be obtained occasionally and the evidence is by no means conclusive. Nor can one agree that "all connections of the celiac plexus with the central nervous system were interrupted."

Other experiments along the same lines have given conflicting results. Hermann and Morin (1), and Morin and Vial (10) found that in anaesthetized dogs bilateral splanchnicotomy alone was sufficient to eliminate the intestino-intestinal inhibitory reflex. Bilateral splanchnicotomy, however, does not always abolish it (17). In unanaesthetized dogs having two Thiry fistulae made from adjacent segments of the upper jejunum, Youmans, Karstens and Aumann (17) found that removal of both lumbar sympathetic chains from above the point where the major splanchnic nerves emerge, eliminated all inhibition in either intestinal segment during distension of the other segment. The same procedure abolished all pain response from intestinal distension. Bilateral vagotomy did not abolish the inhibitory reflex. Ivy and his collaborators (2, 16), on the other hand, have shown that inhibition of bile flow following distension of the colon, persisted in two dogs in which bilateral splanchnicotomy and bilateral excision of the lumbar chains had been performed, and was still present in one of these animals after bilateral vagotomy had been superimposed upon the previous surgical procedures.

One of the chief problems in interpreting the results of all such experiments is the uncertainty that the coeliac ganglia have been completely deprived of all central connections. The only sure method of decentralization is complete sympathectomy, by the method introduced by Cannon, combined with bilateral vagotomy. We have therefore reinvestigated the intestino-intestinal inhibitory reflex in such a preparation. Professor C. W. Hampel has kindly given us advice on the procedure of complete sympathectomy and participated in each operation. Preliminary studies were carried out on animals with only the splanchnic nerves cut, or the lumbar sympathetic chains removed, either on one side or bilaterally. Section of the vagi alone was also carried out in many experiments, but never had any effect on the intestino-intestinal reflex. This observation, merely confirmatory of all previous work, is not included specifically in the results.

#### METHODS

*Recording.* Cats were anaesthetized by nembutal administered intraperitoneally. A loop of jejunum, approximately 15 cm. distal to the point where the small intestine passes behind the colon, was transected together with the attached mesentery down to the large mesenteric blood vessels. A balloon (latex finger cot) was inserted into the proximal segment of small bowel and connected with a tambour for a kymographic recording of intestinal contractions. Another balloon was placed into the distal loop in order to distend the gut. The edges of the opening into the abdominal cavity were clipped together, and the animal was kept warm throughout the experiment. Blood pressure was recorded from the femoral artery. One to two hours were usually allowed to elapse before the experiment was commenced, in order to obtain adequate intestinal contractions. A fairly regular base line

# EXPERIMENTAL INVESTIGATION OF VISCERAL AFFERENT SYNAPSES IN COELIAC GANGLIA

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VISCERAL afferent neurons, for the most part, have their cell bodies in dorsal spinal root ganglia and their first synaptic connections in the spinal cord. The experimental evidence (8, 9, 12, 14) upon which this conclusion is based has been reviewed elsewhere (11, 15). True reflexes involving synaptic transmission take place, according to this view, only through the central nervous system. Certain experimental evidence (13), however, indicates the possible existence of reflexes mediated through 'decentralized' sympathetic ganglia, *i. e.*, those deprived of all connections with the central nervous system. It is thereby implied that within the peripheral sympathetic system are located sensory neurons, the centripetal processes of which end in sympathetic ganglia in connection with postganglionic cells, thus establishing an intrinsic sympathetic reflex arc.

The latter view has received considerable support recently from experiments on the 'decentralized' coeliac and mesenteric ganglia. Kuntz (3) observed the persistence of some synaptic connections in the coeliac ganglia of cats after "the splanchnic nerves were divided and the upper lumbar segment of the sympathetic trunks extirpated bilaterally." Bilateral vagotomy did not appreciably diminish the number of residual synapses. About two weeks were allowed to elapse between the operation and histological study. In other experiments the nerves extending from the coeliac plexus to the gut were sectioned and a few days later some degenerating fibres were found in the proximal parts of these nerves. This observation suggested the presence in the wall of the gut of afferent neurons with fibres running centripetally into the coeliac plexus. Kuntz concluded that the synaptic connections which persisted in the coeliac ganglia, following degeneration of the splanchnic nerves and extirpation of the upper lumbar chains, probably represented the termination of such centripetal fibres. Similar results were obtained in experiments on the inferior mesenteric ganglia (4). The extent of the removal of the sympathetic chains in these experiments is not indicated precisely, which makes it difficult to evaluate the completeness of the decentralization of the ganglia.

Physiological evidence in support of their histological findings was offered by Kuntz and Buskirk (5). In cats inhibition of bile flow follows distension of the distal segment of a transected loop of intestine. The reflex was unaltered by bilateral section of the splanchnic and vagal nerves, a procedure which they refer to as 'decentralization' of the coeliac plexus, despite the fact that the lumbar sympathetic chains were presumably left intact in these experiments. In the same type of preparation inhibition of



left splanchnic nerves remained, intestino-intestinal reflexes could still be produced in all the other animals. As in the group in which bilateral sympathectomy had been performed, it became more difficult to obtain the reflex. Only 40 per cent of a total of 58 distensions were effective. When inhibition did occur, it differed little from that which was found in the other groups, and the latent period remained the same.

*Effect of bilateral splanchnicotomy and bilateral lumbar sympathectomy* Out of 8 cats in which denervation was performed acutely and the inhibitory reflex tested immediately afterwards, inhibition could only be induced in 3 animals, and in these only 16 per cent of 51 distensions were effective. The intestino-intestinal inhibitory reflex was completely absent in 4 chronic preparations, in which bilateral splanchnicotomy and bilateral lumbar sympathectomy had been performed three months earlier.

*Effect of complete decentralization of the coeliac ganglia* In 5 cats both sympathetic chains were removed from above the stellate ganglion down to the level of the brim of the pelvis, in the manner described above. All the splanchnic nerves are thereby divided. Three weeks later the inhibitory reflex was absent in every animal of this series, despite numerous trials. It may be observed in passing that the vagi were still intact at this stage of the experiments. Completion of the decentralization of the coeliac ganglia by bilateral vagotomy had one interesting result. In the intact animal distension of the intestinal loop frequently results in an immediate rise in blood pressure. This vasopressor reflex is still present after complete sympathectomy but is markedly diminished by bilateral vagotomy below the diaphragm. Nevertheless some rise in blood pressure is still perceptible after such decentralization. Presumably the afferent pathway is largely vagal, but somatic nerves in the abdominal wall may participate to a lesser degree. The latter is suggested by the fact that if the loop of intestine to be distended is lifted out of the abdominal cavity, distension no longer produces the reflex in the abdominally vagotomized animal.

## DISCUSSION

The most significant observation of the present series of experiments is the fact that no intestino-intestinal inhibitory reflex could be obtained in any of the completely sympathectomized animals or in those in which bilateral splanchnicotomy and bilateral lumbar sympathectomy had been performed three months earlier. Certainly in the former group there can be no question of any connections, other than vagal, between the coeliac ganglion and the central nervous system. The vagal pathway has been shown repeatedly not to participate in the reflex. The results give no support to the postulated existence of visceral afferent neurons with cell bodies lying in peripherally located ganglia.

Out of 8 cats, however, in which bilateral splanchnicotomy and bilateral lumbar sympathectomy was performed acutely, the inhibitory reflex could be induced occasionally in 3 animals. This difference between the results in

of activity having been obtained, the distal segment of the jejunum was distended by inflating the balloon with 20 cc of air for about 10–15 sec (The volume of air was sometimes increased gradually to 45 cc and for periods up to 40 sec)

*Decentralization of coeliac ganglia* (chronic preparations) Each sympathetic chain was removed in one piece from above the stellate ganglion down to the level of the brim of the pelvis. The entire thoraco-lumbar outflow was thereby eliminated, and all splanchnic nerves, both thoracic and lumbar, divided. The excision was done in three stages. The intestino-intestinal reflexes were tested one week after the last operation. Decentralization of the ganglia was completed at the time of the experiment by dividing the vagus bilaterally, either in the neck or just below the diaphragm. The abdominal vagotomy was in some instances combined with transection of the oesophagus.

In the preliminary studies (mostly acute preparations), four principal pathways had to be considered: the right and left splanchnic nerves and both lumbar sympathetic chains. Only one of these four was cut or removed at any one time, the motility of the intestine was permitted to re-establish itself, and an attempt at inhibition, as outlined above, again tried. This procedure was followed until all four factors were removed. The order in which the nerves were cut or removed was varied in each experiment.

*Splanchnicotomy* The greater and the lesser splanchnic nerves were considered together and cut as they emerged on the under surface of the diaphragm. The peritoneum on the crura of the diaphragm was opened and all the retro-peritoneal tissue stripped away from the muscle in order to be certain that all of the nerves piercing it were interrupted.

*Lumbar sympathectomy* The sympathetic chain was removed from its emergence through the diaphragm down to the level of the brim of the pelvis. The first lumbar ganglia, which are above the diaphragm, placed respectively opposite the vertebral bodies of T13 and L1, are left intact by this procedure.

## RESULTS

*Normal intestino-intestinal reflex* Inhibition of intestinal motility in the proximal loop was produced during distension of the distal loop of intestine in each of 15 intact cats examined. It occurred in 98 per cent of the total 61 attempts at inhibition. The inhibition was characterized by a relaxation of muscle tone, with usually though not invariably, a cessation of intestinal contractions. The latent period was never longer than 3 sec after distension of the distal loop. The duration of inhibition was variable, intestinal activity reappearing in some instances before release of the stimulus, where the latter was maintained for longer periods (30–40 sec).

*Effect of splanchnicotomy* In all 5 animals with unilateral splanchnicotomy, 3 on the left side and 2 on the right, the inhibitory reflex persisted. Inhibition was obtained following 16 of a total of 20 distensions (80 per cent). Bilateral splanchnicotomy was performed in 3 cats, in 2 of which the inhibitory reflex could still be obtained in 12, i.e., 86 per cent of 14 attempts.

*Effect of lumbar sympathectomy* The results were similar in all respects to those found in normal cats. After unilateral sympathectomy the reflex was produced in all of 4 cats investigated. Twenty-one of the 22 distensions were effective (95 per cent). After bilateral sympathectomy inhibition was demonstrated in each of 4 cats so prepared. The percentage of effective distensions fell, however, to 44 per cent of a total of 39 distensions.

*Effect of splanchnicotomy and lumbar sympathectomy, with one of the four nerves remaining* There were 7 cats in this group. The left splanchnic nerves were intact in 3, the right splanchnics in 1, the left lumbar chain in 2, and the right lumbar chain in 1. With the exception of one of the cats in which the

sympathectomy the reflex can rarely be elicited. The procedure may leave some intact connections between the coeliac ganglia and the central nervous system. The possibility of preganglionic axon-reflexes has also to be considered.

5 No support is found for the postulated existence of true reflexes through the decentralized coeliac ganglia.

6 A vasopressor reflex following intestinal distension is still present after complete sympathectomy but is markedly diminished by bilateral vagotomy below the diaphragm. Presumably the afferent pathway is largely vagal, but somatic nerves in the abdominal wall may participate to a lesser degree.

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the acute and chronic experiment of the same nature requires further comment. The most likely explanation is the probability that the coeliac ganglia are not necessarily completely decentralized by the operation. In these preliminary studies no attempt was made to reach the sympathetic chains above the diaphragm, and the upper one or two lumbar ganglia are therefore left intact. One other possibility must be considered.

In 1874 Langley and Anderson (6), confirming an earlier observation by Sokolow, showed that if all the upper branches of the inferior mesenteric ganglia are cut stimulation of the *central* end of one hypogastric nerve results in contraction of the bladder. This does not occur if nicotine is injected intravenously or if the opposite hypogastric nerve is cut. But nicotine does not abolish the effects on the bladder of stimulating the *peripheral* end of the hypogastric, so there was presumptive evidence for a synaptic junction within the inferior mesenteric ganglion. Langley and Anderson then added two significant observations. If one hypogastric nerve is cut and time allowed for nerve degeneration then stimulation of its central end still causes contraction of the bladder with all upper branches of the inferior mesenteric ganglia divided. Thus the nerve fibres stimulated in the experiment cannot be the centripetal processes of visceral afferents whose trophic centres lie close to the bladder wall for these would have degenerated in the central end of the divided hypogastric nerve. Secondly if the section of the upper branches of the inferior mesenteric ganglia is carried out several days before the experiment, allowing time for degeneration, stimulation of the central end of the hypogastric now no longer produces any effect on the bladder. Hence the nerve fibres stimulated in the reflex must have their trophic centre above the inferior mesenteric ganglia. By section of appropriate spinal nerves proximal to the spinal ganglia, Langley and Anderson showed that the actual fibres concerned were preganglionic and probably not posterior root afferent. Further experiments by Langley (7), on similar reflexes in the sympathetic pilot-motor system, led him to establish the existence of collateral branching of all preganglionic fibres, and he designated the type of reflex discussed here as 'preganglionic axon-reflexes'. Some such arrangement might equally explain the difference in results noted here between the acute and chronic experiments.

#### SUMMARY

1 The intestino-intestinal inhibitory reflex can be still obtained after any of the following procedures: bilateral vagotomy, bilateral splanchnicotomy, or bilateral abdominal sympathectomy.

2 So long as one splanchnic nerve or one lumbar sympathetic chain is left intact, the reflex persists.

3 Complete sympathectomy (removal of both sympathetic chains from above the stellate ganglion down to the level of the brim of the pelvis) abolishes the reflex.

4 Immediately after bilateral splanchnicotomy and bilateral lumbar

larly pressure block, caused by arteries below that tolerable minimum. For this purpose, long nerves, cut at a distal level, were threaded through the lumen of a small segment of artery, and then the blocking of conduction of impulses through that region was studied. Hermann (9) ascribes the earliest observations on pressure block of nerve conduction to Fontana (1797). The first work to overcome the technical inadequacies inherent in most of the earlier studies was that of Meek and Leaper (11, contains review of earlier literature), describing reversible block in frog nerves after application of uniform pressures of up to 90 pounds for less than five minutes.

Our own experiments consisted of two groups. In both a limb nerve of a rat was cut and a piece of artery was then pulled for some distance over the proximal stump. In one group, the effects of the sleeve on conduction along the nerve were studied immediately after the operation, while in the other, the examination was not made until the second week after the operation. Nerves prepared in this way were stimulated at the proximal end with "maximal" stimuli, and the resulting action potentials were recorded oscillographically from farther distal levels on either side of the arterial sleeve. Pressure block could thus be identified by the decline and eventual disappearance of the potentials beyond the sleeve. Sleeves which were not naturally constricted could be made to compress the nerve by the local application of adrenalin, and the gradual development of a pressure block could then be followed directly.

#### MATERIALS AND METHODS

A total of 10 white rats was used in the experiments. The sciatic nerve was exposed and either the peroneal or the tibial division was transected at the knee. The transected nerve was freed from its partner as far proximally as could be done without injury. Arteries from small donor animals had been dissected previously and kept in Ringer's solution on ice. Either the carotid or the proximal femoral artery was used. By means of a splicing clutch described previously (16), a segment approximately 5 mm in length was slipped over the proximal nerve stump from its free end. Save for two cases examined immediately, all wounds were closed, and healed without complication. After a lapse of from 8 to 13 days, the operated nerves were again exposed, excised, and placed in a specially constructed stimulation chamber for electrical recording. All operations were done under nembutal anesthesia.

The stimulation cell consisted of a trough of lucite, cca 1 cm wide, 1 cm deep and 14 cm long. At intervals of 5 mm silver wires crossed the trough. In the middle each wire was fashioned into a U-shaped receptacle for the nerve. Save for these bends, the wires were insulated over their full length with "Amphenol 901" cement. From the outside the wires were connected with the binding posts of a multiple selector switch box, the contacts of which were so arranged that by the turning of two dials any combinations of wires could be made to serve as stimulating electrodes and as leads. After its introduction into the chamber, the nerve rested freely on the U-shaped supports of the silver wires in air saturated with moisture.

Action potentials were amplified by means of a condenser-coupled amplifier (time constant 0.01 sec) and were viewed and photographed on the screen of a cathode-ray oscillograph. With this apparatus it was not difficult to recognize repetitive impulses as a standing wave if their voltage was a microvolt or more. Measurements of latency from the stimulus artifact and of peak voltage were made on enlarged projections of the photographic records.

Stimuli were provided by a square-wave stimulating circuit that was triggered by the sweep circuit of the oscillograph. Pulses of less than 0.1 msec duration were employed and were passed through a capacity-shielded transformer to reduce artifacts and render the

# PRESSURE BLOCK IN NERVES PROVIDED WITH ARTERIAL SLEEVES\*

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THE SEARCH for an improved method of re-uniting severed nerve stumps has yielded what appears to be a superior method in the use of arterial segments as splicing agents (15, 16). The cut ends of the nerve are joined without suture inside of a snugly fitting sleeve of artery, which serves the multiple purpose of holding the nerve ends together, preventing fiber escape, branching and neuroma formation, and precluding the ingrowth of fibrous connective tissue detrimental to nerve regeneration. The success of the operation and of the subsequent regeneration depends largely upon the correct fit of the artery. If the artery is chosen too wide for the given size of nerve, it fails to seal the nerve ends, and the space left between tube and nerve invites fibrosis and nerve fiber escape. In order to avoid these hazards, one would feel tempted to use arteries with a lumen narrow enough to clamp down tightly on the nerve ends. Fresh arteries are sufficiently distensible to be fitted over a nerve of considerably larger diameter, so that a firm seal between nerve and artery could be insured. However, it was soon found out that such a tight fit was harmful in that it produced permanent compression of the underlying nerve. Moreover, some arterial sleeves which at the time of operation did not seem to compress the nerve, were at a later time found to have narrowed down appreciably, causing a bottleneck-shaped constriction (see 16, fig. 7). This was never observed when aorta had been used, but was quite common with carotid or femoral arteries, i. e., vessels with a muscular wall. The tightening of the sleeve resulted from a gradual contracture of its wall, which may be ascribed to a direct response of the musculature to pressor substances in the blood. This is the more plausible as vascular muscles after denervation acquire increased sensitivity toward adrenalin (2).

In view of the potential clinical value of the sleeve splicing method, the syndrome of the constricted sleeve deserved more than passing attention. The histological analysis, which revealed a number of interesting facts of more general significance, will be presented in greater detail elsewhere (17). It was the purpose of the experiments reported in the present paper to ascertain the minimum size of artery compatible with undisturbed functioning of the nerve in its interior, as well as the functional disturbances, particu-

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shortness of the exposure. Moreover, the response recorded from a point proximal to the sleeve and fully exposed to the surrounding air likewise failed to recover. The sleeve was removed after 30 minutes (R) with the nerve left in its original position. Records taken 20 minutes later showed no significant recovery of conductivity. The nerve was then removed from the stimulation chamber and placed in Ringer's solution for the next

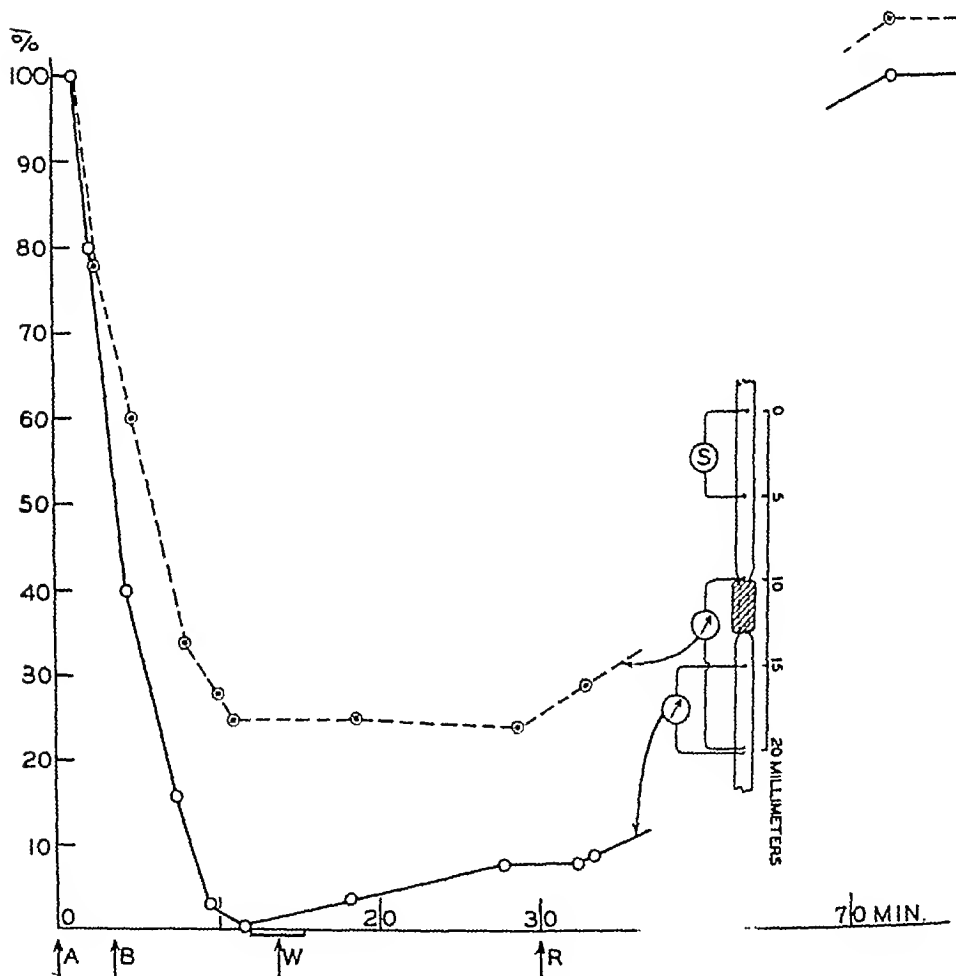


FIG 2 Development of pressure block during adrenalin constriction of sleeve, as revealed by the decline of the amplitude of the action potentials recorded from the points indicated in the diagram, after maximal stimulation at S. Further explanation in text. Ordinates give percentage of the original size of the response at the beginning of the experiment. A, Adrenalin application; B, Blotting; W, Washing; R, Removal to Ringer bath.

twenty minutes. When returned to its original position in the stimulation chamber, it proved to have recovered full conductivity through the formerly compressed region.

The sluggishness with which the pressure block disappeared is attributable to two factors: firstly, even after washing the artery relaxes only gradually, and secondly, the nerve, being relatively plastic and unelastic in its transverse direction, returns to its original diameter but slowly.

Case RB (Fig 3) Segment of carotid artery (3 mm) pulled over tibial nerve. Temperature

stimuli diphasic. The stimuli were always adjusted to a strength slightly but definitely supramaximal for the A fibers. Their adequacy was verified frequently during each experiment.

Five of the tested nerves were prepared for histological sections. They were fixed in Boun's fluid, impregnated with silver according to Bodian, with or without Mallory's Triple Azan Stain.

### EXPERIMENTAL

*A Pressure block by adrenalin constriction of fresh arteries* In these tests oscillographic records were taken immediately after the arterial fragment was slipped over the nerve. Two cases were studied. The nerve was transferred to the stimulation chamber from Ringer's solution and excess fluid was blotted off. The nerve was crushed just proximal to the most distal supporting wire, which also served as one of the recording leads. The positions of stimulating electrodes, arterial sleeve, and recording electrodes are indicated in the diagrams accompanying fig 1 and 2. After constancy of the response had been ascertained, a drop of adrenalin (1:1000) was placed on the arterial sleeve, and as soon as a diminution of the action potential spike beyond the sleeve became noticeable, a series of photographs was started. The elevation of the peaks of the response over the base line was used as an index of the volume of nerve fibers conducting through the sleeve. For easier comparison, percentage values instead of absolute values were used in making the graphs, with the amplitude of the "maximal" response at the beginning of an experiment set at 100 per cent.

*Case RA (Fig 1 and 2) Segment of carotid artery (3 mm) slipped over tibial nerve. Temperature 23.5°C.* Records were taken alternately from a point distal to the sleeve (solid line) and a point near the proximal end of the sleeve (broken line). At both points, the action potentials dropped rapidly after adrenalin was applied (A). The reduction expresses the progressive interruption of conduction in the nerve fibers at the level of the sleeve. It is not due to a possible shunting effect of the drop of adrenalin placed upon the nerve, for neither does the application of the drop itself produce a decrease of the amplitude, nor does the blotting off of the drop (at B) arrest further decline. Eleven minutes after adrenalin administration conduction of impulses through the area of the sleeve has practically ceased. Even at the highest amplifications, there was no evidence of impulses passing through the compressed area. The pressure block has become complete. Records from the area proximal to the sleeve have, in the meantime, dropped to about one-fourth of their original size.

In order to test the reversibility of the block, the sleeve was rinsed with Ringer's solution while remaining on the electrodes (W). At first, the response returned to only about 10 per cent. Either the sleeve had failed to relax its grip on the nerve, or the nerve fibers had been permanently damaged, or finally, the constricted nerve segment being shut off from the medium by the sleeve, had become asphyxiated. The last explanation may

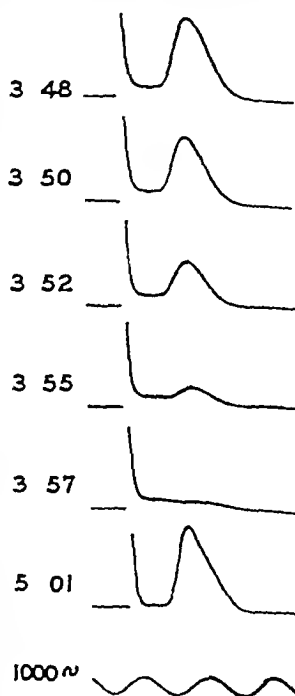


Fig 1 Decline and recovery of oscillographic response recorded from nerve distal to constriction sleeve after adrenalin application and washing. Adrenalin was applied at 3 48. The times at which records were taken are indicated on the margin. Further details in text.

be discounted in view of the



Damage to some of the nerve fibers along the surface diminished the distal response as compared with the earlier determinations. In the graph, Figure 3, second half, the size of this response was set as 100 per cent. Adrenalin was then applied. As can be seen from the graph, the pressure block developed much more slowly than it did either in the same preparation on the previous day, or in case RA. Even 26 minutes after the first (A-), and 15 minutes after the second adrenalin administration (A<sub>2</sub>), the response distal to the sleeve had lost only 61 per cent of its amplitude. This slowness can be explained by the fact that a considerable number of fibers had been ripped off, so that the nerve was thinner than on the preceding day, rendering the arterial grip less effective.

Otherwise, case RB corroborates case RA in showing that a nerve which was exposed to a local compression block with complete interruption of conduction may gradually regain its conductivity after being released from the compressing agent. That the conduction block is due to the compression by the sleeve rather than to a direct effect of adrenalin, can be considered as certain. Not only has adrenalin never been shown to have any such blocking effect on nerves, but it has failed in our own experiments to affect conduction when placed upon the nerve directly.

The reason for the reduction of the response in the proximal nerve portion immediately adjoining the sleeve is not entirely clear. Above all, we do not know whether it indicates an actual drop in the number of conducting fibers or an increase in the shunting within the nerve, which reduces the fraction of the total action potential recordable from the surface, or possibly a distance effect of the kind observed by Gerard (6) after local nerve asphyxiation. Tangl (14) and Stroebe (13) both have observed that strong compression of a nerve forces axonal substance back into the uncompressed portions of the fibers. This might cause an ascending disturbance of conductivity. However, it is doubtful whether the volume of fibers blocked proximal to the actual constriction would be large enough to account for a decline of such magnitude as was registered at this level (e.g., in case RB, within 5 minutes a 12 per cent decline 6 mm proximal to the sleeve, Fig. 3, black dot). This leaves as main explanation of the proximal decline the fact of increased shunting.

One must bear in mind that all the interstitial liquid has been squeezed from the sleeve and thereby added to the liquid content of the nerve on either side. Since, owing to the shunting effect of extraneuronal fluid, the volume of action potential recordable from the surface varies with the ratio of axonal to non-axonal nerve content, any such increase of interstitial liquid for a given fiber volume means a reduction of the recordable fraction of generated potential. The increase in fluid content of the nerve near the constriction can actually be recognized as a slight bulbous swelling, and histological studies of chronic cases show the presence of voluminous edemas (Fig. 4, 5). It, therefore, seems reasonable to ascribe most of the observed reduction of the amplitude of the records at some distance from the compression to a registration artifact rather than to real interference with conduction.

*B Cases with chronic constriction.* Table 1 lists the 8 cases in which an arterial sleeve has been present over the nerve for from eight to thirteen days prior to the oscillographic tests. In six of them (R45, R47A, R47B, R48, R50, R51) impulses were found to be conducted no farther than the proximal end of the sleeve. No trace of conduction into and through the sleeve was ever noticed, even at highest levels of amplification. In all these cases the artery, when first placed over the nerve, fitted tightly. In the days following the operation, it had undergone further contraction, a fact which became clear from the histological preparations.

Lack of conduction through the sleeve raises the question of whether there was merely a physiological pressure block or whether the nerve had undergone degeneration. Thus, five of the six specimens were sectioned and studied histologically. The impregnation of one (R51) was unsatisfactory, the remaining four showed various degrees of degeneration, which may be briefly described.

25°C Adrenalin was put on the sleeve ( $A_1$ ) and the excess fluid was sponged off three minutes later. Eleven minutes after the application of adrenalin, the response has dropped to 10 per cent of its initial size. The nerve was then washed by filling the whole stimulation chamber with Ringer's solution for three minutes (W). Records taken after the fluid had again been pumped out showed that conduction through the sleeve had not improved and that the response proximal to the sleeve, which had dropped to slightly over 40 per cent, had likewise held its level. The bath in Ringer's solution was then repeated for another three minutes but there was no perceptible change in the response. The artery was still

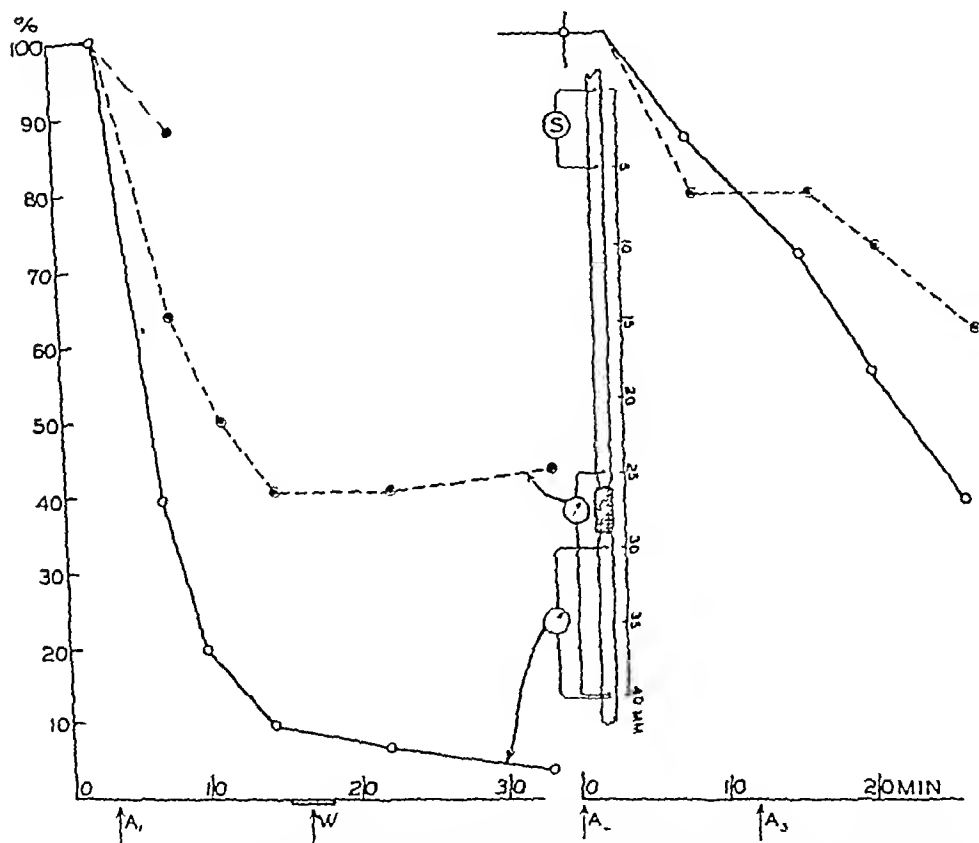


FIG 3 Decline of the oscillographic response after adrenalin application ( $A_1$ ) and constriction of the sleeve and recovery after prolonged washing. Symbols as in Fig 2. Further explanation in text.

tightly clamped around the nerve with the total diameter of artery plus enclosed nerve still slightly smaller than that of the free nerve alone. Thirty-five minutes after the beginning of the experiment, the artery was stripped off. The resistance encountered in this manipulation proved that the sleeve was still actively contracted, and its mark on the nerve could be seen in the form of a conspicuous constriction. This region was then washed again thoroughly, but only an exceedingly small response had returned at 40 and 42 minutes.

The nerve was then transferred to Ringer's solution and placed in the ice box. The next morning, it was remounted in the stimulation chamber. Stimulation thresholds were found to be approximately the same as on the previous day and good responses were obtained at all points through the area of the former constriction. The temperature was 23.5°C. The arterial sleeve was then again slipped on to its position of the previous day.

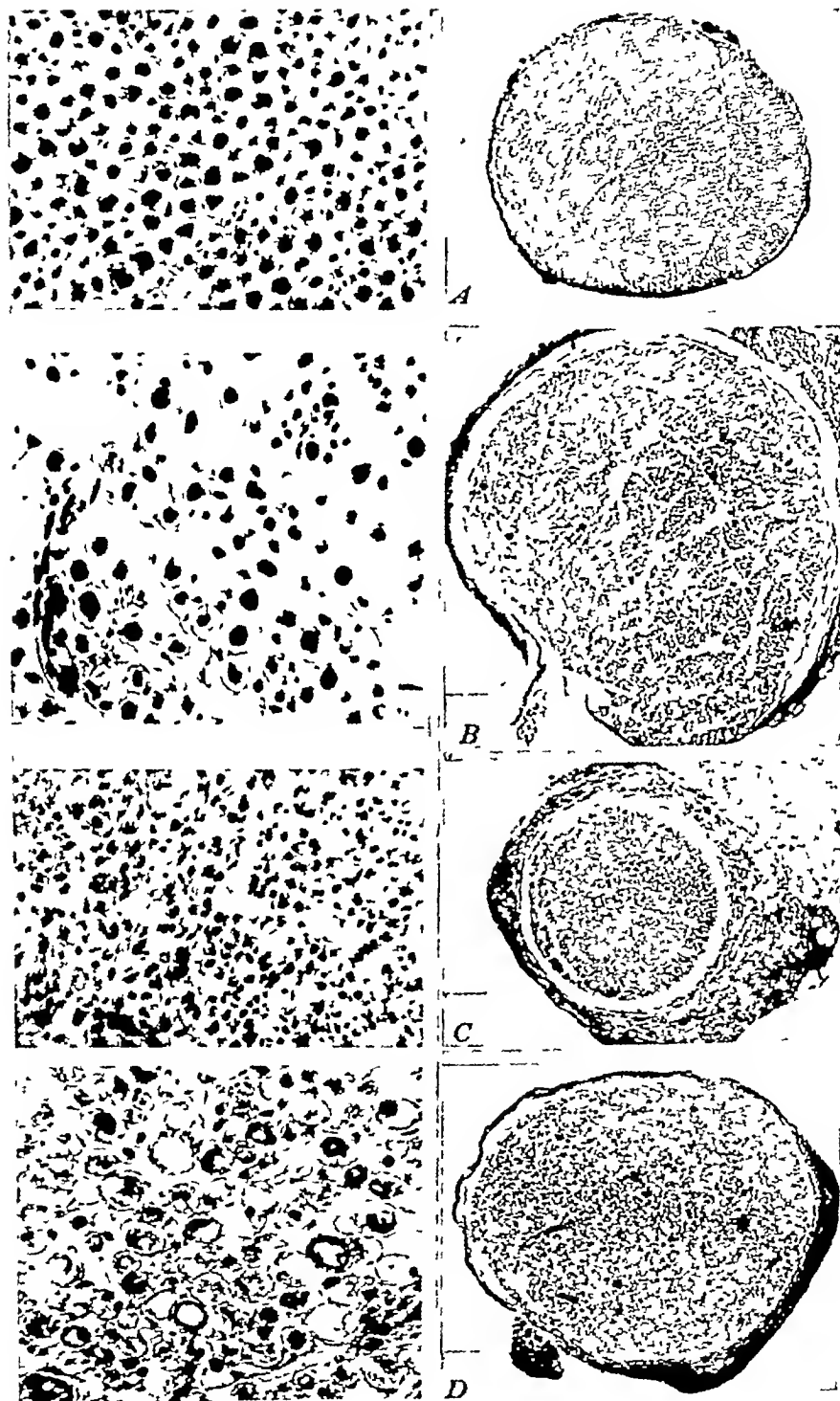


FIG 4 For legend see opposite page

In general, the histological appearance of these nerves duplicates some of the features described in spliced and regenerated nerves with constricted sleeves kept for much longer periods (16). These are (i) edema of the nerve proximal to the constriction, (ii) swelling of the axons proximal to the constriction, (iii) tight packing and loss of diameter of the nerve fibers within the compression zone. As these features are discussed in greater detail elsewhere, they may be illustrated here merely by a sample case. Figure 4 shows cross sections through the nerve of specimen R50, 10 days after putting on the sleeve. The levels from which these sections were selected are indicated in the diagram, Fig 5. All sections being photographed at an identical magnifica-

Table I

Specimen	Host weight	Nerve	Artery			Examined after days	Conduction Block			Histology	
			Name	Weight of donor gr	Fit		Edema	Permanent	After adrenalectomy	Sections	Degeneration
45	210	Per	Fem	30	Tight	12	+	+		Long	Complete
46	210	Per	Fem	220	Loose	13	-	-	+	—	—
47A	180	Per	Fem	65	Tight	10	++	+		Long	Complete
47B	180	Tib	Car	65	Tight	10	++	+		—	—
48	180	Per	Fem	60	Tight	10	+	+		Long	Partial
49	180	Per	Car	80	Close	8	-	-		—	—
50	180	Tib	Car	80	Tight	10	++	+		Cross	?
51	180	Tib	Car	80	Tight	10	++	+		Negative	—

tion, the variations in the diameter of the nerve become at once evident. Moreover, the graph, Fig 5, gives the actual values of cross sectional area, as determined by planimetry of camera lucida drawings, plotted over the length of the nerve.

Figure 4A illustrates the almost unaffected proximal portion of the sciatic nerve, one recognizes the internal division of the nerve into a peroneal and a tibial branch, the latter being the experimental nerve to be followed in the subsequent figures. In the region of the edema, which begins about 3 mm proximal to the sleeve, the nerve is swollen to nearly twice its proximal diameter (Fig 4B), owing to the accumulation of large amounts of fluid in the endoneurial spaces. This edema indicates, as is discussed more fully elsewhere (17), that there occurs in the endoneurial spaces a persistent centrifugal seepage of fluid. The constriction of the sleeve, by obliterating the endoneurial channels, causes a damming up of this fluid in front of the bottleneck, thus swelling the nerve—in the present case, to about three times its original cross sectional area. The appearance of the nerve inside of the sleeve (Fig 4C) is in marked contrast to that of the edematous region. The fibers are tightly packed and there are few endoneurial spaces left. All fibers are of small diam-

from undegenerated to predominantly degenerated portions is fairly abrupt. At levels peripheral to the sleeve, the proportion of intact fibers is rather

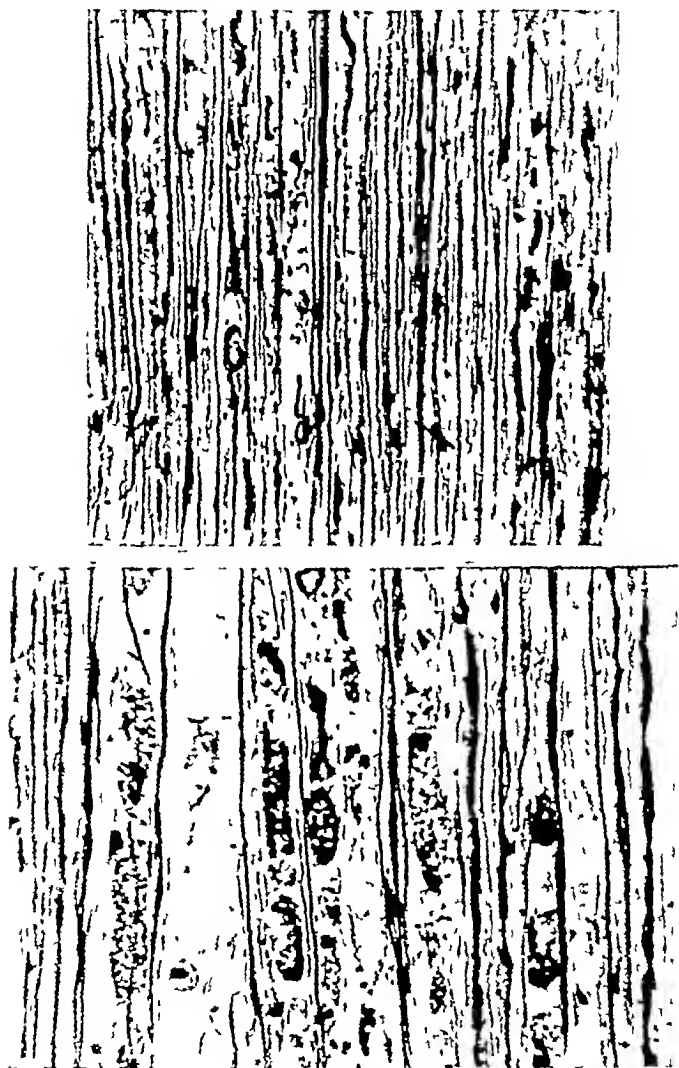


FIG. 6 Photomicrographs of longitudinal sections through the compressed (top) and the farther distal portion (bottom) of the nerve, R47A. Note the number of intact fibers, mostly of small caliber, in the sleeve region (top), and the presence of numerous intact fibers of small and medium caliber, as well as of some regenerated fibers, amidst remnants of degenerating fibers in the peripheral portion (bottom).

small. Moreover, in animals R45 and R48 all fibers present are extremely fine, and there is every reason to assume that they represent regenerated rather than the original branches. This they reveal by occasional branching, certain

eter Degenerating fibers, scarce at the entrance into the sleeve, appear in increasing numbers as one proceeds distally. At the exit from the sleeve, the nerve suddenly widens again and reaches a cross sectional area of about 50 per cent above normal (Fig 4D). This peripheral swelling is definitely not due to an edematous condition, as the nerve fibers are contiguous and there is no excess interstitial fluid. Its main cause is the extensive degeneration going on in this area, the individual degenerating axons being transformed into swollen tubes of Schwann. The slight flanging of this portion, shown in Fig 5, may be an elasticity effect. In our cases, true edematous swelling invariably appeared at the proximal, but never at the peripheral side of the constriction.

Many axons are widened at the level just proximal to the bottleneck, narrowing suddenly to the smaller caliber observed inside the sleeve. As will be shown in a subsequent paper, this phenomenon indicates that the column of axonal substance is subjected to a steady centrifugal pressure or flow.

The histology of the other 4 cases presents essentially the same picture. The only variation lies in the different proportions of intact fibers in the region beyond the sleeve. This can be better estimated in longitudinal than in cross sections. Three of the nerves, R45, R47A, and R48, have been sectioned longitudinally. All three have in common that many fibers within the compressed area are intact, with the axons continuous, of smooth contour, and well impregnated with silver, that as one proceeds peripherally, more and more fibers in various stages of typical Wallerian degeneration come into view, and that the transition

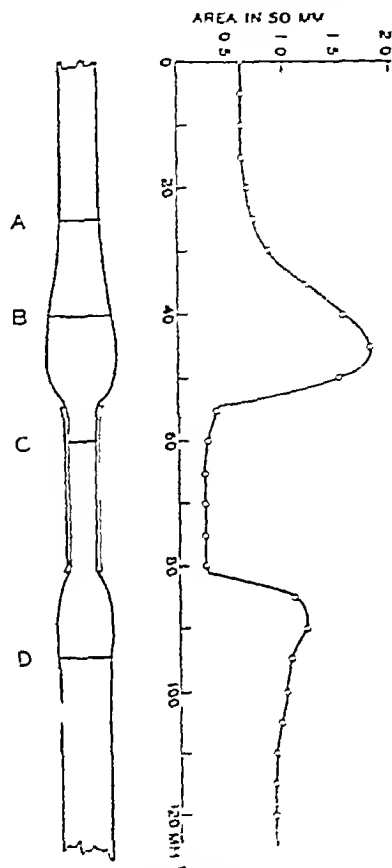


FIG 5 Diagram of nerve, R50, constructed from measurements of the diameters at consecutive levels. In the right half of the figure, total cross sectional area is plotted over the length of the nerve from planimetric determinations made at 0.5 mm intervals.

FIG 4 Cross sections taken at four different levels of a tibial nerve constricted by a sleeve of artery (R50). The dimensions of the nerve and the positions from which the cross sections were chosen are indicated in correct proportions in the diagram, Fig 5.

A, intact part of sciatic nerve, tibial division (experimental nerve) at left

B, edematous portion of tibial nerve

C, compressed region

D, degenerating peripheral part

Representative portions of each section are reproduced under higher magnification at the left.

were adjusted in the graph to the same relative strength as the simultaneous value of the distal record, *i.e.*, 88 per cent. After renewed adrenalin application ( $A_2$ ), it took less than ten minutes for the distal response (at the 25 mm mark) to decline again to about 20 per cent. Simultaneously, a less extensive decline was recorded from points 20 and 15 mm. After renewed washing ( $W_2$ ), the response from beyond the sleeve failed to recover appreciably and the experiment was discontinued.

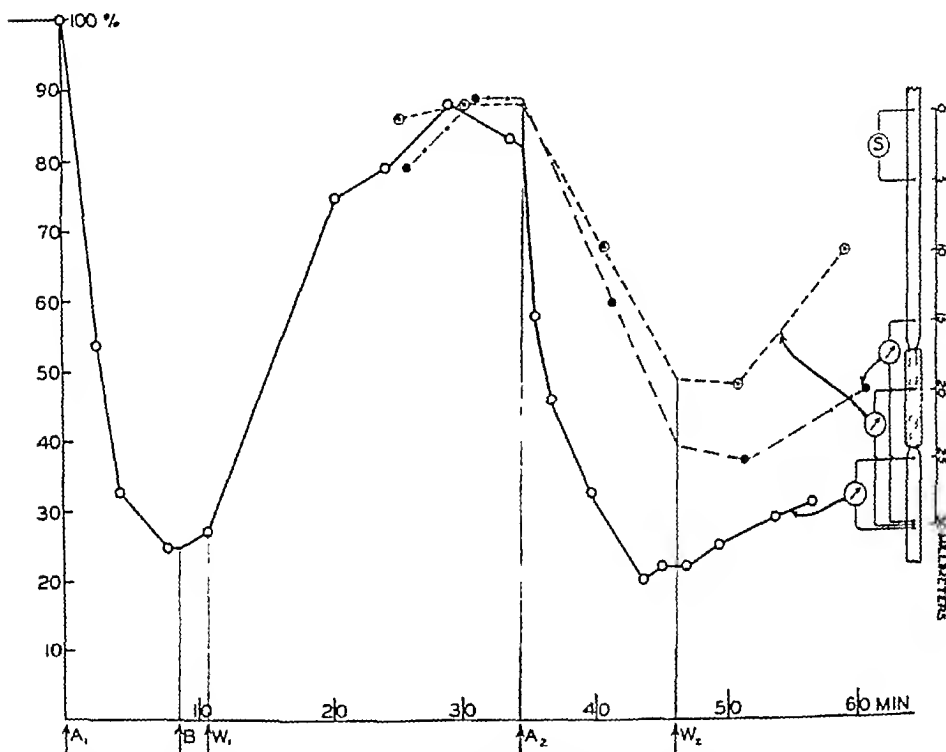


FIG 7 Decline and recovery of action potential during adrenalin constriction and relaxation of an arterial sleeve transplanted 13 days previously. Electrode position indicated in diagram. Symbols and units as in Fig 2 and 3. Further explanation in text.

The following conclusions can be drawn from this experiment. The transplanted segment of artery was still responsive to adrenalin after 13 days in the body. The time course according to which pressure block developed was of the same order in the fresh and in the transplanted artery. In contrast to experiments RA and RB, the first Ringer's bath produced a fairly rapid recovery of conductivity through the sleeve region. This may be ascribed either to the fact that the adrenalin was washed off before the constriction had reached its full extent, or to a slight weakening of the arterial wall after transplantation, permitting the nerve to redistend the relaxing artery and recover its former diameter sooner. At any rate, the experiment proves that a pressure

irregularities of their course and their position relative to the degenerated Schwann tubes. On the basis of a most conservative estimate of regeneration speed of 2 mm per day (8) the 10 to 12 days available to these fibers after the operation would have been ample for regeneration through the 5 to 6 mm stretch beyond the end of the sleeve, which is all there was in our preparations.

In these two cases, the apparent extinction of the impulses in the sleeve region is, therefore, readily explained by the absence of sufficiently large and numerous nerve fibers to give a recordable response. The fine newly regenerated fiber branches were imbedded in such a mass of non-axonic shunting tissue that their potentials may well have been lost before reaching the recording electrodes.

In contrast, the third case, specimen R47A, contained a much larger proportion of intact fibers in its peripheral part, and most of these could be recognized as old rather than regenerated fibers. This is indicated by their position and, above all, size, some being of medium caliber with distinct sheath (Fig. 6). Since the volume of these intact fibers passing through the sleeve into the peripheral region was large enough to have yielded a fair action potential, the fact that no trace of such activity was discernible proves that these fibers were physiologically blocked in the compressed region.

Prolonged pressure block may, therefore, exist without destroying the integrity and continuity of the nerve fiber. The fact that only one in three cases has given this result in our series, clearly indicates that the margin of pressure between one which merely blocks the nerve physiologically and one which leads to morphological degeneration is not very wide.

*C. Adrenalin constriction in transplanted sleeves.* Two cases, R46 and R49, failed to show pressure block, and conduction through the sleeve region was wholly unimpaired. The sleeves in these cases were marked at the time of the operation as fitting "loosely" or "closely" rather than "tightly." Lack of constriction was demonstrated by biopsy. Table 1 shows that these two arteries were, relatively speaking, the largest of the set. In R46, the sleeve came from a donor of the same size as the host, and in R49, it was transplanted from an 80-gram donor to the peroneal nerve of a 180-gram host. As is shown by cases R50 and R41, the same size of artery over a tibial nerve would produce pressure block, but the tibial at this level is about twice the size of the peroneal.

Conduction through the sleeve of nerve R46 being perfect, this specimen was used to test the effectiveness of adrenalin in sleeves transplanted for nearly two weeks. The experiment was done in the same manner as described for cases RA and RB. Figure 7 shows the results (temperature 23° C).

Seven minutes after adrenalin application ( $A_1$ ), the response has dropped to 25 per cent of the initial size. After rinsing with Ringer's solution ( $W_1$ ), the response recovered to 88 per cent. After that, records were taken not only from points distal to the sleeve but also from within the sleeve and proximal to it, as indicated in the diagram. For the purpose of easier comparison of subsequent relative changes, the second readings from the proximal points



dicates that the response declines in proportionate steps within successive intervals. The initial steepness of the decline can be attributed to the fact that the large caliber fibers are the ones to succumb to pressure block first (5).

Since the pressure within the sleeves must, for physical reasons, distribute itself evenly throughout the compressed area, the localization of the actual block must be explained in terms of differences in sensitivity of the nerve fiber to the acting pressure. In case R46, for instance, many fibers conducted more than half way through the compressed zone. Other fibers are blocked farther proximally. Denny-Brown (personal communication) finds that the first morphological changes under continued pressure occur at the nodes of Ranvier. In view of the fact, however, that nerve fibers with definite signs of Wallerian degeneration may still be capable of conducting impulses (10), a precise localization of the point of pressure block from purely morphological criteria may not be possible.

A question of considerable importance is whether or not nerve compression produces an actual change in the size of the axon. In the chronic experiments of our series, the nerve was compressed to less than half its original size (see Fig 5 and 6). If such a diminution were to be achieved merely by the obliteration of the endoneurial spaces without reduction of the size of the axons, at least half of the nerve cross section would have to consist of endoneurial spaces. In the rat this is definitely not the case, and part of the observed shrinkage in our cases must, therefore, be ascribed to actual decrease in the diameter of the fibers. Our histological studies seem to bear out this contention. Nerves which have been under compression for from one to two weeks show total absence of larger fibers even in the most proximal parts of the sleeve where little degeneration has occurred (see Fig 4C and 6). If the large fibers had been destroyed, their remnants would be plainly visible. Thus the only valid conclusion seems to be that the continued compression has reduced the fiber diameters to such an extent as to bring them all into the small or medium caliber class. Similar observations have been reported by Cajal (1, p. 302) after moderate ligation of nerves.

The fact that the arterial constriction has produced physiological pressure block on the one hand, and morphological degeneration of the fibers on the other, might suggest that a common alteration of the nerve underlies both phenomena. This view is contradicted, however, by the fact that a fair proportion of nerve fibers have been able to survive a pressure block of ten days duration (case R47A). At the end of this period, the fibers were intact morphologically, yet incapable of conducting impulses across the compressed area. Thus, then, proves that the local change which blocks transmission in the fiber does not necessarily entail degeneration of a physiologically isolated peripheral portion. A prediction to this effect was made by Cook and Gerard (4), and the same conclusion must be reached from numerous clinical observations of nerves which have become paralyzed by the pressure of scar tissue or other constrictions. In some instances, the mere

block of short duration is promptly reversible, although after a second adrenalin constriction ( $A_2$ ), washing failed to have the same restorative effect as before. The response recorded from within the sleeve region (Fig 7, broken line) declined markedly less than that recorded from beyond the sleeve (to 49 per cent as compared with 18 per cent), which reveals that at least half of the fibers, even at the height of the compression, were not blocked until in the distal portion of the sleeve. The decline of the response at the 15 mm mark (2 mm proximal to the sleeve) can again be ascribed to the shunting effect of nerve fluid squeezed from the compressed area.

### DISCUSSION

According to these experiments, an arterial segment pulled over a nerve may behave in three different ways, depending on its caliber. If wide enough, it may leave the nerve permanently undisturbed. If of medium width, it will not interfere with the nerve under ordinary circumstances but will clamp down and produce pressure block of varying intensity when exposed to adrenalin. If of smaller diameter than the nerve itself, it will produce permanent compression, resulting in loss of conductivity and, in more serious cases, degeneration of the nerve distal to the constriction.

In studying the development of the physiological pressure block oscillographically, we have tacitly ascribed any decline in electric response registered from the nerve beyond the constriction to the extinction of impulses in some of the fibers at the level of the block. This assumption, however, needs qualification. As illustrated in Fig 5, compression by an arterial sleeve may reduce the nerve in its lumen to less than half its former size. Under this lateral compression, the interstitial fluid of the endoneural spaces is displaced longitudinally and squeezed into the parts of the nerve bordering on the compressed area. As this augments the non-axonal shunting material in the nerve, a certain reduction of the recordable action potential in this region should be expected. The size of any decline referable to this source can be estimated from the records taken proximal to the sleeve (dotted lines in Fig 2 and 3). Comparing these values with the drop recorded distally, one realizes immediately that no more than a minor fraction of the latter can be ascribed to shunting.

Within 10 minutes after the initiation of the constriction of the sleeve by adrenalin, the passage of impulses through the constricted area may have become completely abolished. That the block of transmission is due to the compression, and to no other influence, is clear. Neither adrenalin applied to the nerve directly without a sleeve, nor the presence of a sleeve without adrenalin has any similar effect on the nerve. The sleeve may be left on the nerve for a considerable length of time without interfering with conductivity, yet upon applying adrenalin, the pressure block appears within several minutes.

Our graphs (Fig 2, 3, 7) reveal a systematic trend in the development of the pressure block. They resemble logarithmic functions, which would in-

- 13 STROEBE, H Experimentelle Untersuchungen über Degeneration und Regeneration peripherer Nerven nach Verletzungen *Beitr path Anat* , 1893, 13 160-278
- 14 TANGL, F Zur Histologie des gequetschten peripheren Nerven *Arch mikr Anat* , 1887, 29 464-470
- 15 WEISS, P Re-union of stumps of small nerves by tubulation instead of suture *Science*, 1941, 93 67
- 16 WEISS, P Nerve regeneration following tubular sphcing of severed nerves in the rat *Arch exp Surg* , 1943, 46 525-547
- 17 WEISS, P Endoneural edema in constricted nerves *Anat Rec* , 1943, 86 (in press)

operative removal of the scar tissue brought almost instantaneous relief of the paralytic condition and resumption of conduction in the nerve, a condition which could not possibly have been obtained if the blocked fibers had been in a state of degeneration. Likewise, some reports in the literature about crushed nerves quote time values for the recovery far too brief to have allowed for regenerative outgrowth of new fibers from the crushed area to the end organs. One would suspect, therefore, that in these cases, while the paralysis after crushing was complete, not all fibers may have been actually interrupted, the intact ones merely suffering from temporary pressure block.

The occurrence of degeneration of the peripheral parts of fibers within constricted sleeves indicates that the constriction has produced a trauma as severe as if the fiber had been transected or crushed. That Wallerian regeneration may occur even without the opening of the neurilemmal sheath has, of course been known from previous studies on ligated nerves (1, 13) and from the observations on beri-beri (3), and our present cases are merely another illustration. On the positive side, however, they give us little information about the actual cause of the degeneration. We cannot entirely exclude the possibility that ischemia may have been involved inasmuch as the strangling action of the sleeve shuts the peripheral nerve end off from circulation for as much time as is needed for collateral vascularization to become established. This, however, takes only a few days, and it is questionable whether this could cause degeneration. It is much more likely that the compression has arrested the centrifugal flow inside the axon of substances or factors vital for the maintenance of axonal integrity, much along the lines of Gerard's (7) and Parker's concept of degeneration (12). This flow can apparently still proceed in a state in which impulse conduction along the fiber is locally blocked. Thus we must conclude from the cases of partial degeneration with complete pressure block.

It is interesting to note that the symptoms of degeneration were much more evident in the nerve distal to the sleeve than within the sleeve itself, where the actual compression was taking place. This corroborates more extensive observations by Denny-Brown (personal communication). Cajal (1, p. 292) likewise notes that in ligated nerves the parts of the fibers directly under the ligature fail to break down. The fact is not easy to explain unless it were by the assumption that the pressure has not only paralyzed the conductive mechanism but also those properties of the nerve fiber and of the accompanying sheath cells which are responsible for the thorough transformations after trauma which we commonly call degeneration. We know that degeneration is an active process involving cell activities, and not a merely passive breakdown. It is possible that these progressive changes were inhibited either by lack of space or by interference of pressure with a more subtle mechanism.

In practical respects, these experiments underscore the importance of the correct choice of artery for nerve splicing. The choice must consider both the size of the lumen in proportion to the nerve caliber and the amount

- 13 STROEBE, H Experimentelle Untersuchungen über Degeneration und Regeneration peripherer Nerven nach Verletzungen *Beitr path Anat*, 1893, 13 160-278
- 14 TANGL, F Zur Histologie des gequetschten peripheren Nerven *Arch mikr Anat*, 1887, 29 464-470
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# NOTE ON THE ORGANIZATION OF THE TACTILE SENSORY AREA OF THE CEREBRAL CORTEX OF THE CHIMPANZEE

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IN A RECENT PAPER on the cortical representation of cutaneous tactile sensibility in the monkey (7) an analysis of the pattern of cortical organization in terms of metameric segmentation was presented. The analysis showed (Fig. 3) that the spinal segments from T<sub>1</sub> through the last caudal (Ca<sub>1</sub>) are projected to the contralateral postcentral gyrus and paracentral lobule in the same serial order as that in which they are arranged in the cord and with overlapping at the cortical level comparable in degree to that seen in the corresponding dermatomes. The serial order of the cervical segments is retained, but on projection to the cortex these segments are reversed *en bloc*. This reversal brings the cortical fields of the upper cervical segments in contiguity with the cortical fields of the upper thoracic segments, and places the cortical field of C<sub>1</sub> adjacent to that of the trigeminal nerve.

It was also pointed out that the reversal of the cervical projection results in two regions of metameric discontinuity in the cortical sensory sequence, that between the cortical fields of the trigeminal nerve and C<sub>8</sub> and that between the upper cervical and the upper thoracic fields. It was noted that the boundaries formed by these regions of metameric discontinuity apparently coincide with the division lines separating the face, arm and leg areas as defined by Dusser de Barenne's (2) strychnine method.

The study on which the analysis was based showed that the cortical sensory sequence in the monkey differs in several important respects from that currently accepted for man (3,5). It appears probable that these differences may be accounted for by the greater difficulty of obtaining the necessary data on man rather than by a real phylogenetic variation. Evidence in support of this view may be found in the important, but little noted, paper by Sittig (6) on sensory fits in man. For a discussion of Sittig's observations see our paper on the monkey.

The purpose of the present note is to report certain observations which we were privileged to make on the chimpanzee "Dayton" after Dr. Marion Hines\* (4) had explored the motor region of the precentral gyrus with electrical stimulation. Although few, the observations indicate that the sensory sequence in the chimpanzee is similar to that described for the monkey.

\* We wish to thank Dr. Hines and Dr. A. H. Schultz for the opportunity of examining this animal.

## MATERIAL AND METHODS

The animal, an adult male (*Pan leucoprymnus*), 15 years old, weighing 47.17 kg, was received under ether anesthesia at the end of a day of experimentation in the Department of Anatomy (May 26, 1938). In the Department of Physiology the ether was gradually supplanted by intravenous pentobarbital-sodium. The animal survived about 4 hours under the latter anesthetic. Conditions were not as favorable for observation as in monkeys under pentobarbital-sodium and the period of survival was not long enough to permit examination of many points on the postcentral gyrus.

The methods of study were the same as those used on the monkey (7). Cortical responses were evoked by mechanical movements of hairs on various parts of the body surface or by light pressure applied to the skin. The cortical responses thus evoked were amplified electrically and visualized on a cathode ray tube.

## OBSERVATIONS AND COMMENTS

The character of the cortical response evoked by moving a few hairs on the posterior aspect of the left heel near the edge of the sole may be seen in

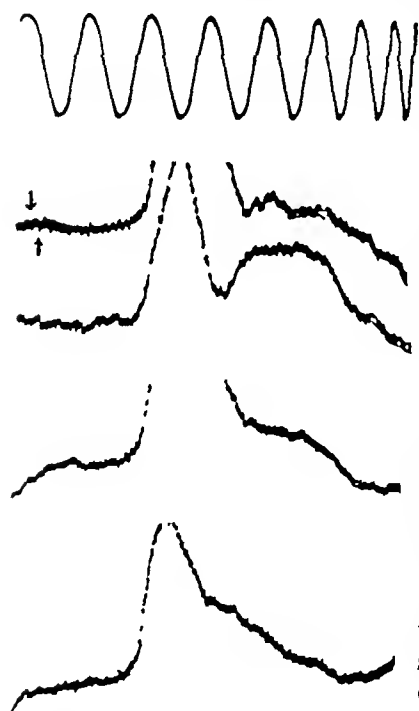


FIG 1 Four cathode ray oscillograph traces of surface positive cortical responses evoked at HEEL point on postcentral gyrus (Fig 2) by movement of a few hairs on posterior aspect of contralateral heel. Arrows at left indicate beginning and end of stimulus. Time signal 60~/sec.

the examples given in Fig 1. The responses were surface positive and consisted of a relatively fast component of 22 to 30 msec duration, which began approximately 26 msec after application of the stimulus, and a slower wave of lower amplitude which ended nearly 100 msec after stimulation. Since the standing height of the animal, as measured by Dr. Schultz, was 129 cm, the overall conduction velocity of the fastest elements was approximately 50 meters per sec.

This type of cortical response was seen at all points examined on the postcentral gyrus when the appropriate portion of the contralateral cutaneous surface was stimulated. Figure 2 shows the loci of the 7 points studied. Each of these has been labeled according to the peripheral area which, when stimulated, gave largest responses at that point. The labels on the precentral gyrus show the arrangement of the motor foci as determined for this animal by Dr. Hines (4).

Two features of the sensory arrangement are of special interest. (1) The point labeled HEEL was found to receive impulses principally from the heel and the lower posterior part of the leg—a cutaneous field which lies on the postaxial aspect of the hindlimb. (2) Impulses from ear, occiput and anterior aspect of the neck entered the

cortex high up on the postcentral gyrus at the level of the precentral trunk area. The cutaneous area concerned is supplied by nerves from upper cervical segments. These two findings are significant and indicate that the sensory sequence in the chimpanzee is similar to that described for the monkey. The fact that the heel point in the chimpanzee is on the dorsal aspect of the

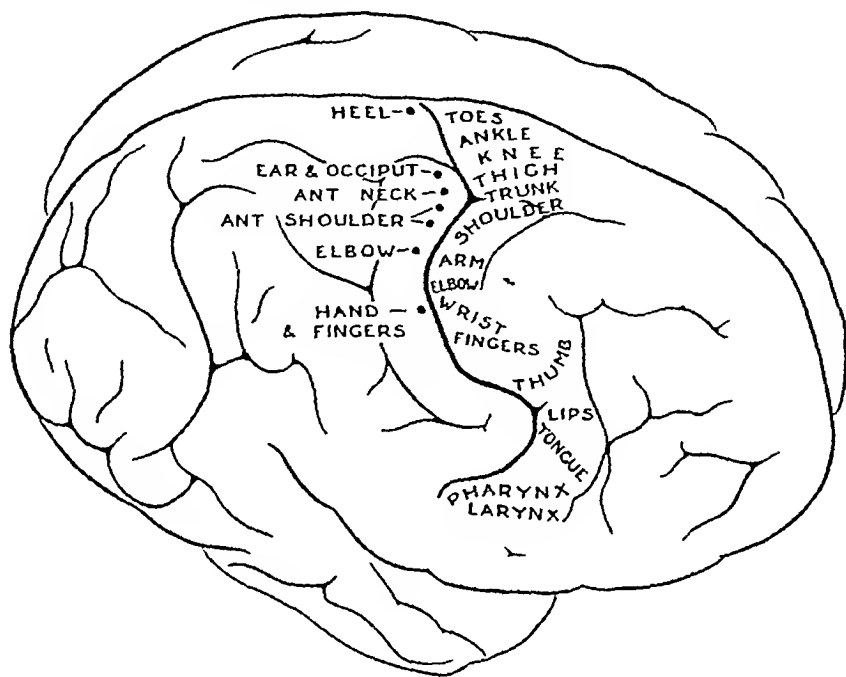


FIG 2 Drawing (X1) of dorsolateral surface of the right cerebral cortex of chimpanzee "Dayton". Dots on postcentral gyrus mark points at which surface positive electrical responses were evoked on tactile stimulation of various portions of the contralateral cutaneous surface. Each dot is labeled to indicate the cutaneous area which, when stimulated, gave rise to largest potentials at that cortical point. Labels on the precentral gyrus show the pattern of motor localization in this animal as determined by Dr. Marion Hines (4). We wish to thank Dr. Hines for permission to reproduce her original figure with modifications.

hemisphere suggests that the sensory area for the toes is also on the dorsal surface. (Compare with Expt. X, [7].)

The relation of these observations on the chimpanzee to the pattern of representation in the monkey can be seen with the aid of Fig. 3, which represents a frontal section through the postcentral gyrus and paracentral lobule of the monkey. The sensory sequence is given by the labels placed along the surface of the cortex. Note the location of foci for occiput, ear, side of head and neck, and for the heel. The latter is on the medial aspect of the hemisphere. The analysis of the cortical arrangement in terms of metamereres is shown by the overlapping heavy lines. Labels on the afferent projection fibers indicate the spinal segments to which they are related. The broken



lines at the boundaries between the fields of the trigeminal nerve and  $C_8$  and between  $C_2$  and  $T_1$  correspond to the boundaries between the face, arm and leg areas of Dusser de Barenne (2) In the chimpanzee, the projection area for the upper cervical nerves (occiput, ear, side of neck, shoulder), as indi-

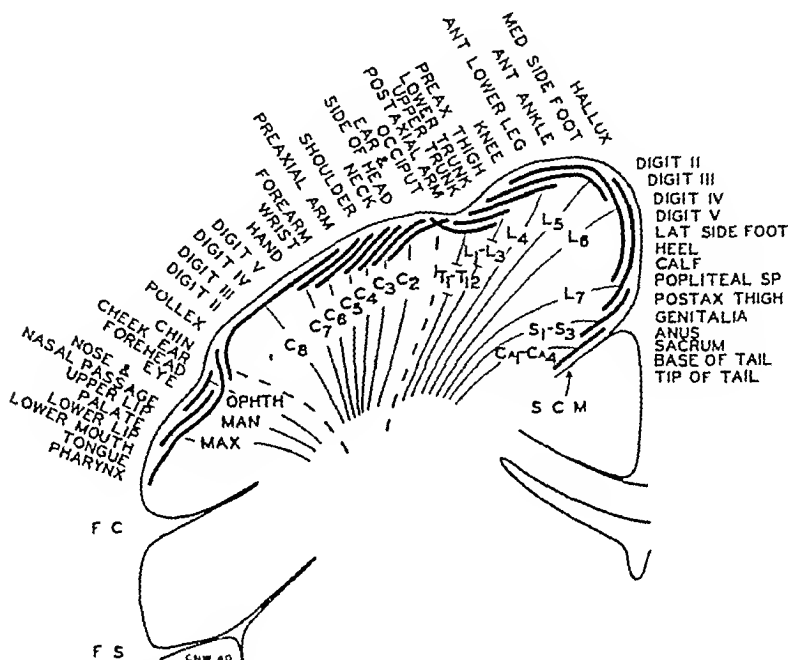


FIG 3 Diagram to scale of frontal section through the postcentral gyrus and paracentral lobule of the monkey, giving the sensory sequence and showing, according to our analysis, the serial arrangement on the cortex of the dermatome projection and the overlap of cortical areas for successive dermatomes. Note the reversal of the cervical projection as compared with the projection of segments  $T_1$  through  $Ca_4$ . The broken lines at boundaries between the cortical areas for the trigeminal nerve and  $C_8$  and between  $C_2$  and  $T_1$  correspond with the boundaries between the face, arm and leg areas of Dusser de Barenne (2). F S, Sylvian fissure, F C, central fissure, S C M, callosomarginal sulcus, MAX, maxillary division of n. trigeminus, MAN, mandibular division, OPTH, ophthalmic division, C, cervical, T, thoracic, L, lumbar, S, sacral, Ca, caudal (From (7)).

cated by our observations, lies adjacent to the boundary between the arm and the leg areas as defined for this species by Dusser de Barenne, Bailey, Garol and McCulloch (1). It is apparent, therefore, that this boundary line in the chimpanzee, as in the monkey, coincides with the region of metameric discontinuity (between  $C_2$  and  $T_1$ ) produced by *en bloc* reversal of the cervical spinal segments on projection to the cortex.

#### SUMMARY

Although few, the observations here reported indicate that the tactile sensory area is organized in the same way in the chimpanzee as it is in the monkey. In the latter, analysis of the cortical pattern in terms of metameres

showed that all spinal cord segments below C<sub>8</sub> are projected to the cerebral cortex in their spinal sequence, whereas the cervical segments on projection are reversed *en bloc*. This produces two regions of segmental discontinuity in the cortical sequence. These regions coincide with the boundary lines separating Dusser de Barenne's face, arm and leg areas.

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# NEURON PATTERNS CONTROLLING TRANSMISSION OF IPSILATERAL HIND LIMB REFLEXES IN CAT

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THE SEGMENTAL REFLEX discharge (7, 31, 21) must be considered of anatomical rather than functional significance in that it contains, in unnatural combination, those elements which constitute the several distinct ipsilateral reflexes. In the present paper are the results of experiments designed to resolve the segmental reflex into its functional components. The observation that a major division of the segmental reflex into its direct (two-neuron-arc) and indirect (multineuron-arc) components followed segregation of muscle afferent and cutaneous afferent fibers for afferent stimulation (21) provides the point of departure for the experiments to be described. Some of the present observations have been mentioned briefly in a preliminary note (23).

A general discussion of these and other results will be found in another paper (25).

The afferent fibers of the A group (14) exhibit a range of diameters extending from  $20\mu$  to  $1.5\mu$  (36). In a dorsal root the whole range of fibers is present, but in the peripheral nerves significant segregations are found (36, 8, 29, 14) which permit a degree of selective stimulation of the various components (21). For the purposes of the present discussion the afferent fibers will be classified into groups, each group being marked by a peak in the fiber distribution plots of one or another of the several peripheral nerves. Group I consists of the largest afferent fibers, which are to be found only among the afferent fibers arising from muscle. Approximately these fibers range from  $20\mu$  to  $12\mu$  in diameter (8, 29), with a distribution peak at 15 to  $16\mu$ . Group II contains fibers of approximately  $12\mu$  to  $6\mu$  in diameter, with a mode at 8 to  $9\mu$ . These fibers form a prominent peak in the fiber distribution plots of cutaneous nerves (8, 30, 14), but they are poorly represented among the muscle afferent fibers (8, 29). Group III consists of fibers gathered about a peak at 3 to  $4\mu$  (the delta pile). These last are to be found in both muscle and cutaneous nerves. Another category, to consist of the C fibers, the afferent and reflex function of which is proven (3, 2), should be included as group IV. These fibers have not been studied during the course of the present experiments.

Since group I and group II fibers are the lowest threshold fibers in muscle and cutaneous nerves respectively, they may be excited in isolation by the simple expedient of selecting the appropriate nerves for stimulation (21). There is no means at present of stimulating group III fibers in isolation but their contribution to reflex action, on stimulation, is easily recognizable as addition to the reflex discharges caused by stimulation of the larger, lower threshold fibers [after-discharge? ] (45).

The experiments were performed on cats, made spinal by transection accomplished through the dorsal atlanto-occipital membrane under ether anaesthesia, after which artificial respiration was instituted and the anaesthetic discontinued

*Group I reflexes* The reflex discharge resulting from stimulation of group I afferent fibers has been studied chiefly, but not exclusively, in connection with the nerve supply to the gastrocnemius muscle. Several considerations prompted this choice, not the least among which is the fact that the afferent fibers from this muscle have been examined by histological means (8, 29). Moreover, the gastrocnemius muscle is supplied through the seventh lumbar (L7) and first sacral (S1) segments of the spinal cord, which provides a favorable site for study by virtue of the length of the nerve roots pertaining to those segments

Figure 1A illustrates the reflex discharge, recorded from the S1 ventral root following stimulation of the nerves of the gastrocnemius muscle. The

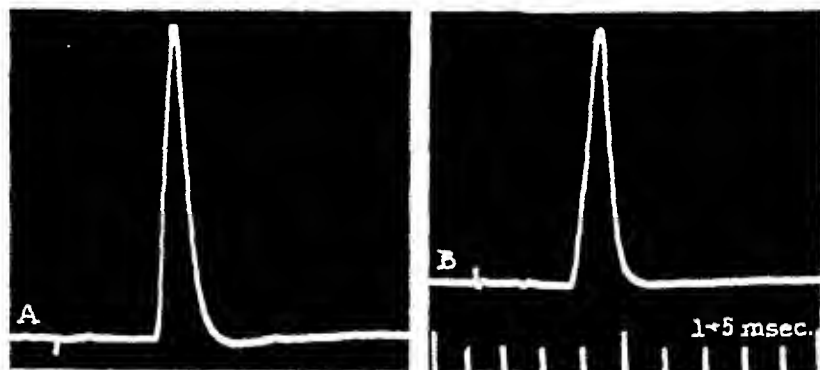


FIG. 1. Group I reflex. A—reflex discharge recorded from the S1 ventral root on stimulation of the gastrocnemius nerves. B—reflex discharge recorded from the gastrocnemius nerves on stimulating the S1 dorsal root. Time in 1 and 5 msec intervals. In all figures where there are two time designations these are for the small and large divisions respectively.

ventral roots supplying the muscle were severed distally in order to record the reflex and to prevent the penetration of the inevitable antidromic volley into the spinal cord. Under these circumstances the gastrocnemius nerves are connected with the spinal cord only through the dorsal roots, and may be regarded therefore as 'afferent' nerves. Figure 1B presents the reflex discharge obtained by stimulating the S1 dorsal root while recording from the gastrocnemius nerves. The dorsal root was severed distally to prevent the dorsal root volley from coursing antidromically into the gastrocnemius nerves. Under these circumstances the gastrocnemius nerves may be regarded as 'purely efferent' in function. The reflex discharge from the dorsal root to the gastrocnemius motor fibers is essentially similar in latency and duration to that from the gastrocnemius afferent fibers to the ventral root. The conduction length of the reflex pathways is similar for the experiments illus-

trated in Fig 1A and B, and so therefore are the central delays. The reflex discharges in Fig 1 have a latency of approximately 2.6 msec, which is appropriate for reflexes transmitted through arcs of two neurons, considering the overall length of the pathways involved. Figure 2 presents an estimate in greater detail of the central delay of a reflex discharge comparable to that illustrated in Fig 1A.

The gastrocnemius nerve to S1 ventral root reflex latency for the experiment illustrated in Fig 2 is 2.5 msec. In the inset (B) of Fig 2 the conduction time for the long afferent limb of the reflex is recorded by the use of leads, one placed on the dorsum of the spinal cord at the root entry zone of the (intact) S1 dorsal root, the other on nearby cut bone. By measurement, afferent conduction to the spinal cord requires 1.4 msec. By subtraction, 1.1 msec is required for conduction in the intraspinal course of the dorsal root fibers, for synaptic delay, and for ventral root conduction. Of these events ventral root conduction accounts for approximately 0.3 msec. The remainder, 0.8 msec, may be designated as central delay, i.e. synaptic delay and central conduction.

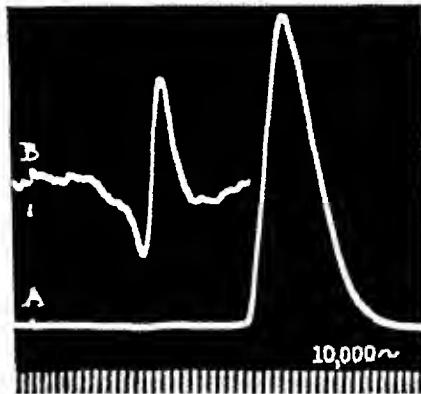


FIG 2 Group I reflex. A—reflex discharge recorded on S1 ventral root on stimulation of the gastrocnemius nerves. B—afferent volley evoked by stimulation of the gastrocnemius nerves and recorded from the dorsum of the spinal cord at the root entry line. The difference in latency of A and B measures the sum of central latency and ventral root conduction time for the reflex discharge. Time 10,000 cycles.

A central delay of this order of magnitude forces the opinion that arcs of two neurons are involved (27, 31). The total duration of the reflex spike-potential is 1.45 msec, the total conduction distance approximately 19 cm, the band of fibers active extends approximately from  $20\mu$  to  $12\mu$ . If the duration of the single axon spike is taken as 0.5 msec (14), the total reflex has a dispersion of approximately 0.95 msec. Now, at 19 cm conduction, a volley, initially synchronous, would have, on travelling in a group of fibers varying from  $20\mu$  to  $12\mu$  in diameter, a dispersion of 1.05 msec calculated by using the conversion constant of 6 proposed by Hursh (17) to derive conduction velocity from diameter. Granting that some or all of these figures may be approximations, the dispersion encountered in experiment with the reflex discharge is within the limit calculated for simple transmission along an equivalent nerve bundle. There is, in consequence, reason to suppose that the reflex two-neuron-arc pathways alone can account for the group I reflex discharges that have been described.

The two-neuron-arc discharge reflects into the stimulated muscle nerve. When stimulating and recording leads are placed on the same nerve, with all central connections to the spinal cord intact, a two-neuron-arc discharge may be recorded, as illustrated in Fig 3A. The stimulated volley courses

centrally in both afferent and motor fibers, and since these fibers are in general similar in the case of the group I reflexes, the reflex afferent volley and the antidromic volley will reach the spinal cord essentially in simultaneous combination. Only a small fraction of the reflex volley under these circumstances (22), however, intermediate strengths of stimulation can be found which provide a sufficiently large afferent volley to produce a reflex without blocking too many of the reflex pathways by virtue of the antidromic volley. The use of a large nerve trunk facilitates the adjustment of the stimulus to attain this end.

At this point a note relative to the dorsal root reflex is in order. In many of



FIG 3 Group I reflex. A—reflex volley stimulated and recorded on the tibial nerve. B—the same stimulation after transecting the L7 and S1 dorsal roots of the same side to confirm the reflex nature of the discharge. The 'tail' of the directly conducted volley is seen in both A and B. The time relations of the reflex volley show that it is transmitted through arcs of two neurons. Cf. also Fig 9, 10 for other examples of two-neuron-arc reflex transmission into the stimulated nerve.

the present experiments afferent fiber pathways between the stimulating and recording leads exist to provide potential paths for the transmission of dorsal root reflexes to the recording leads. This is the case particularly when recording from the stimulated peripheral mixed nerve (44). As the central latency of the dorsal root reflex is 4 msec (44), it does not interfere with the recording of group I reflexes, but it may appear in the nerve later to mimic other true reflex discharges. In practice, the temperature of the preparation is maintained as near normal as possible to minimize the dorsal root reflex.

In Fig 3A is shown the reflex discharge through two-neuron-arc pathways as recorded on the tibial nerve on stimulation of that nerve. The reflex discharge follows by approximately 4.0 msec the volley conducted directly from stimulating to recording electrodes. The added latency for this reflex over that found when the reflex is recorded on a ventral root (Fig 1, 2) is approximately 1.5 msec, which is just sufficient to account for the added efferent conduction distance from the ventral root to the recording leads on the tibial nerve (cf. also Fig 9A, G for a similar reflex discharge recorded from another preparation). Figure 3B shows that the two-neuron-arc discharge is removed by section of the appropriate dorsal roots. After section

of the dorsal roots there may be a slight residual discharge, which is due in part to a recurrent or 'pseudo-reflex' volley from the central regions of the motoneurons as a result of the uncurtailed antidromic volley (32, 22), and in part probably to discharges arising in the manner of the Hering phenomenon at the cut ends of the dorsal roots (15, 34, 20), possibly by the action of negative after-potential. Whatever the residual discharges may represent, Fig 3B provides the essential control to show that the centrifugal volley in Fig 3A is a true reflex volley rather than a recurrent volley of similar time relationships (32, 22).

The two-neuron-arc reflex discharge does not reflect into muscle nerves other than the one stimulated. In Table 1 is to be found a list of reflex pathways from one nerve to another which have been searched for two-neuron-arc

*Table 1 Reflex pathways without two-neuron-arc reflex discharges*

Stimulated nerve	Recorded nerve	Remarks
Gastroc lat	Gastroc med	Fig 4B and C
Gastroc med	Gastroc lat	Fig 4E and F
Gastroc (med and lat)	Tibial (less gastroc)	Fig 4H and I Fig 8B and C
Tibial (less gastroc)	Gastroc (med and lat)	Fig 4K and L
Peroneal	Gastroc	No reflex or very small delayed discharge, possibly a dorsal root reflex
Gastroc	Peroneal	Fig 7F, G, H, I Group III reflex, sometimes group II reflex
Sciatic (less gastroc)	Gastroc	No reflex discharge
Hamstring	Gastroc	No reflex discharge
Tibial	Peroneal	Fig 4M, 9 Group II and group III reflexes
Peroneal	Tibial	Very small late discharge, possibly dorsal root reflex or residual ipsi-lateral extensor discharge
Superficial peroneal	Deep peroneal	Late discharges
Tibial	Tibialis anterior	Fig 10 Late discharges
Tibial	Deep peroneal	Fig 11 Late discharges
Peroneal (less tibialis anterior)	Tibialis anterior	Late discharges Group II reflexes

reflex discharges to no avail, together with references to the several figures containing illustrative records.

Figure 4 illustrates the absence of reflex discharges from one to another of the divisions of the tibial nerve, even at strengths of stimulation calculated to recruit all the A fibers of the stimulated nerve into the 'afferent' volley. Record M of Fig 4, for which the tibial nerve was stimulated while recording from the peroneal nerve, serves as a control for the viability and patency of the central portions of the reflex system in the experiment illustrated. It will be noted that the discharge recorded in 4M has a latency of 5.8 msec, whereas the two-neuron-arc discharge should appear, as in Fig 3, with a latency approximating 4.0 msec.



Particularly interesting among the observations of Fig. 4 is the fact that no reflex is obtained as between the nerves to the two heads of the gastrocnemius muscle. At this juncture one should recall the experiment originally performed by Sherrington (41, 42), and confirmed by Liddell and Sherrington (18) and again by O'Leary, Heinbecker and Bishop (29). In each case the effect of stimulating the central end of the severed nerve to one head or

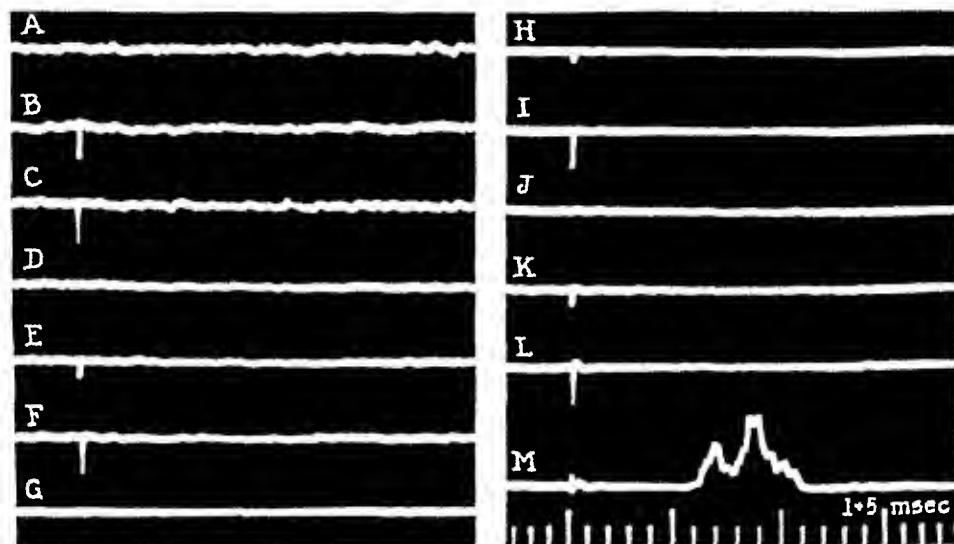


FIG. 4 The two-neuron-arc discharge is not transmitted to muscle nerves other than the one stimulated (cf. also Table I and Fig. 7, 8, 9, 10). Group II and group III reflexes are not recorded ordinarily from extensor nerves (cf. also Fig. 8). A, D, G, J—blank sweeps with recording leads on the gastrocnemius med., gastrocnemius lat., tibial, and gastrocnemius nerves respectively. B, C—weak and strong stimulation of gastrocnemius lat. N recording gastrocnemius med. N. E, F—Similar to B, C but stimulating and recording leads interchanged. H, I—stimulation of N. gastrocnemius recording on N. tibial (less its gastrocnemius branches). K, L—similar to H, I, but stimulating and recording leads interchanged. M—stimulation of tibial N recording from peroneal N—group II reflex.

fraction of a muscle (gastrocnemius or quadriceps) was inhibition of the innervated remainder of that muscle, gauged on a background of decerebrate rigidity or crossed extensor reflex. At the time these experiments were performed it was not realized that the antidromic volleys unavoidably transmitted centrally to the central portions of the motoneurons could effect the transmission of reflex effect through neighboring motoneurons not involved in the antidromic volley (32). The effect, usually inhibitory, is particularly potent as between motoneurons supplying parts of the same muscle. There can be little doubt that this action of antidromic volleys accounts, in good measure, for the observations of Sherrington, Liddell and Sherrington, and O'Leary, Heinbecker, and Bishop. Of course, the possibility of direct inhibition in the orthodromic sense (19, 21) cannot be neglected but the crucial experiment has not yet been devised to demonstrate direct orthodromic inhibition in this situation.

In spite of the known antagonism between the reflex arcs to the several heads of a muscle, and the fact that this antagonism is exerted when the interacting volleys arrive at the motoneurons in concert, the action cannot contribute significantly to the absence of two-neuron-arc discharges from one head to another within a muscle (Fig. 4B, C, E, F) or from one nerve to another (Fig. 4H, I, K, L, 7, 8, 9, 10, 11) for the depressant action of antidromic volleys is not great for the two-neuron-arc reflex at the time relationships which obtain in the present experiments, *i e* (virtually simultaneous combination of the afferent and antidromic volleys at the spinal cord)

From the experiments described it is possible to conclude that the afferent limb for the mediation of two-neuron-arc reflex discharge consists of the large, low threshold group I afferent fibers arising in muscle (*cf* also 21), and that the two-neuron-arc discharge reflects only into the muscle group of muscles or head of a muscle, the afferent fibers of which are stimulated. Of course it is reasonable that, on the occasion of further subdivision of a muscle nerve twig, a stage might be reached, perhaps fortuitously, in which two-neuron-arc discharges could be obtained by stimulation of one subdivision while recording from another. The endeavor to achieve this state of subdivision has not been pursued.

One cannot escape the identity of distribution that obtains between the two-neuron-arc reflex discharge and the myotatic reflex (18). In effect the two-neuron-arc connections appear to constitute the pathway for mediation of the myotatic reflex. The relatively synchronous discharge evoked in this pathway by single shock stimulation would then imitate the phasic response to stretch (*i e*, the tendon-jerk) in its most brief, and possibly unattainable (*cf* 12, 24) form. Further direct evidence for this position is to be found in another paper (24).

*A comparison of group I and group II reflex effect.* While the local reflex effect attending stimulation of group I fibers is confined to two-neuron-arc pathways, the reflex discharge following stimulation of a cutaneous nerve or of the medium threshold fibers (cutaneous for the most part) of a mixed nerve, has all the attributes of the multineuron-arc discharge as encountered in the segmental reflex (21). Furthermore, the distribution of the reflex evoked by stimulation of the cutaneous afferent fibers is quite different from that of the group I reflex.

In Fig. 5 are compared the effects of stimulating group I and group II fibers, as recorded from the dorsum of the spinal cord after the manner of Gasser and Graham (13) and of Hughes and Gasser (16). Figure 5A characterizes the events on stimulation of the nerve to the medial head of the gastrocnemius muscle, the appropriate ventral roots being severed. Recorded with the same electrode positions, Fig. 5B illustrates the events on stimulation of the sural (external saphenous) nerve. Fig. 5C shows the recorded result of combined stimulation of the two nerves. The conduction distance from the stimulating electrodes to the spinal cord was so arranged as to be equal in the two nerves. Study of Fig. 5 reveals that the group I

afferent volley is recorded from the dorsum of the spinal cord as a triphasic variation (*cf* 13) practically devoid of an associated and ensuing negative intermediary potential, whereas, in contrast, the smaller group II spike potential is followed immediately by a prominent negative intermediary potential, signalling internuncial activity.

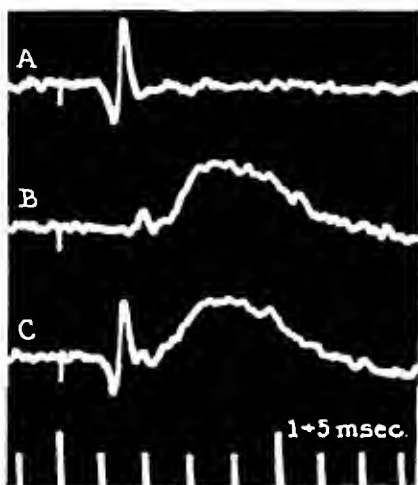


FIG 5 Records from the dorsum of the spinal cord A—the nerve to the medial head of the gastrocnemius muscle is stimulated B—stimulation of the sural nerve C—combined stimulation of the gastroc med N and the sural N Note longer conduction time for sural nerve volley than for gastroc med nerve volley, and negative intermediary potential evoked by sural nerve stimulation

In 5C, on the occasion of simultaneous stimulation of the two nerves, all the elements occurring severally in records A and B are present in approximate summation, which indicates a rather high degree of independence between the two reflexes under the conditions of the experiment (*i.e.*, with synchronous stimulation) and among the elements that contribute to the cord potential as recorded from the cord dorsum (but *cf* Fig 12 for interaction at another interval of shocks. Comparison of Fig 5, 6 and 12 illustrates the fact that the reflex effect of the cutaneous nerve stimulation, the flexor reflex, is prepotent)

Figure 5 emphasizes the different conduction characteristics of the group I and group II afferent volleys. The difference in latency of conduction amounts to approximately 0.4 msec with conduction distance of 12 cm. It is important, therefore, to make corrections for differential afferent conduction velocities when comparing the time relationships of group I

and II reflexes, the correction value of course increasing as the afferent limb of the reflexes is increased in length.

The reflex discharges evoked by stimulation of group I and group II afferent fibers, and as recorded from a ventral root are quite different (21). Figure 6 shows such reflex discharges, both severally and in combination. Record A of Fig 6 shows the group I reflex on stimulating the gastrocnemius nerves. Records B, D, F, H, illustrate the group II reflex similarly recorded in isolation, and resulting from stimulation of the sural nerve. There is random variation in the response from one observation to another. In records C, E, G, I the two reflexes are combined by synchronous stimulation of the gastrocnemius and sural nerves. The conduction distances are equal. Again, there is variation from one observation to another, but there is apparently no systematic change to suggest that the transmission of the gastrocnemius (*i.e.*, extensor) two-neuron-arc discharge has had any definite effect, one way or the other, on the succeeding discharges. The reflex evoked by sural nerve stimulation is directed into flexor channels (43). It is an important consider-

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will be seen on comparing Fig 5 and 6 that the group I reflex transmitted in its entirety is not accompanied by activation of the internuncial elements contributing to the cord potential (13, 16) On the contrary, the group II afferent fibers when stimulated yield intense activity among the interneurons of the dorsal regions of the spinal cord, which in turn causes the diffuse delayed discharges characterizing the group II reflex Figures 7, 8, 9, 10, 11, 12 reveal that the group II multineuron-arc discharges are distributed overwhelmingly to the flexor musculature, as would be expected from the early observations of Sherrington (43) These facts provide ample confirmation for the association, developed by Hughes and Gasser (16), between the cord potentials and the flexor reflex

*The distribution of group II and group III reflexes* The peroneal nerve, considered as a 'motor' nerve, is distributed in the main to muscles of physiological flexion (43) In contrast, the tibial nerve contains motor fibers distributed to posterior tibial and plantar muscles, muscles of physiological extension As an approximation these nerves may be considered as flexor and extensor nerves respectively, and in practice, no essential distinction, in terms of recorded reflex discharges, has been found between the parent trunk of the peroneal nerve and its constituent branch to the tibialis anterior muscle on the one hand, or between the parent trunk of the tibial nerve and its constituent branches to the gastrocnemius muscle on the other hand With these considerations in mind, the distribution of activity engendered by stimulation of group II and group III afferent fibers has been examined

Separation of the group II and group III fibers, the latter comprising essentially the delta fibers, depends primarily upon the strength of stimulation Figure 7 illustrates the reflex responses recorded from the peroneal

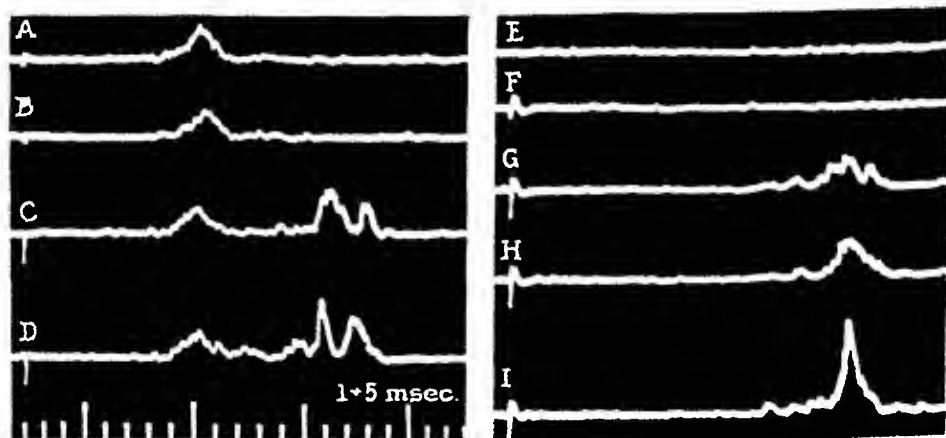


FIG 7 A, B, C, D—stimulation of sural nerve A, B—stimulation at group II strength C, D—stimulation at group III strength Recording leads on peroneal nerve E—blank sweep F, G, H, I—stimulation of gastroc nerves recording leads to peroneal nerve F—stimulation at group II strength—no reflex G, H, I—stimulation at group III strength—group III reflex

ation that the extensor two-neuron-arc reflex and the flexor multineuron-arc reflex evoked by a single *synchronous* stimulation are virtually independent, for this combination will occur on stimulation of a mixed nerve such as the tibial nerve even though only the flexor discharges are recorded, as when the recording leads are directed to the peroneal nerve (*cf* Fig 9) With combined stimulation, the two-neuron arc discharge is unhindered for, travelling in afferent fibers of the highest velocity, it finds the spinal cord in the 'resting' state

Observations made from the dorsum of the spinal cord (Fig 5) and from a ventral root (Fig 6) under similar circumstances invite comparison It

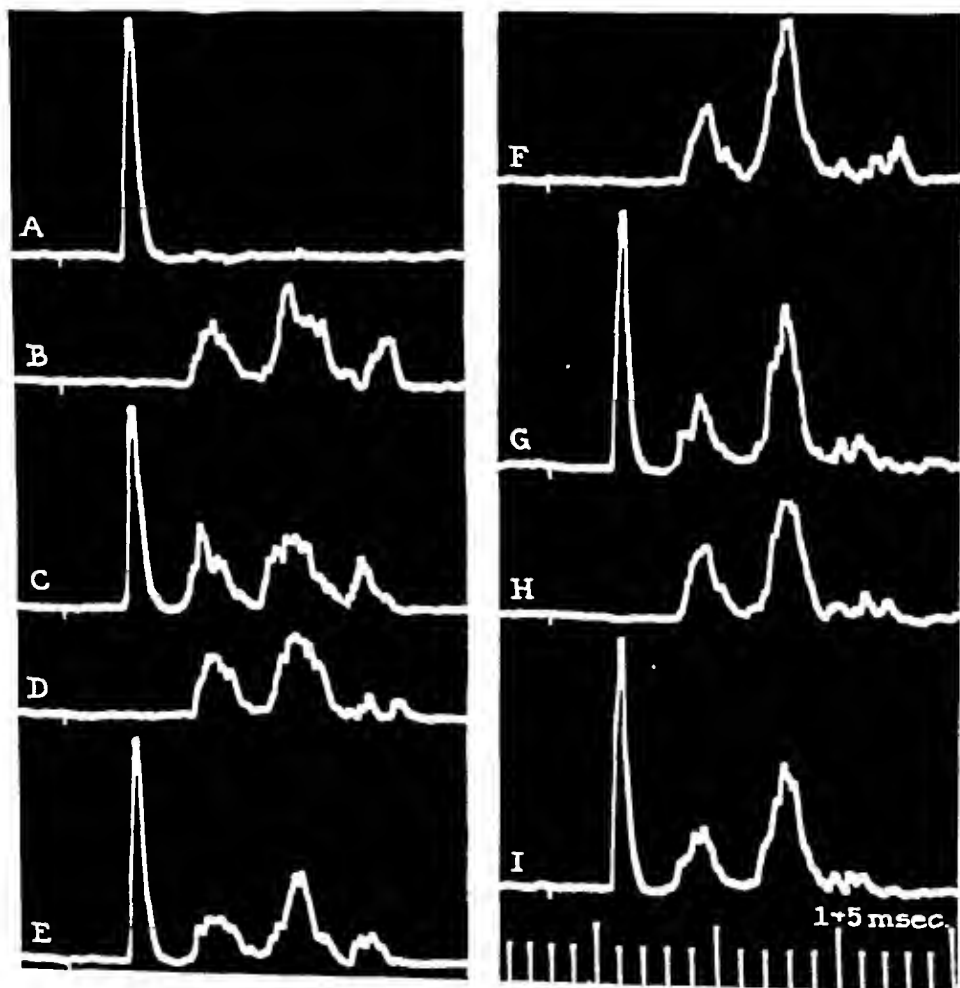


FIG 6 Records from the S1 ventral root A—stimulation of gastroc nerves—group I reflex B, D, F, H—stimulation of sural nerve—group II reflex C, E, G, I—combined stimulation of gastroc and sural nerves The discharge of the group I reflex appears to have no systematic effect of the group II reflex which follows

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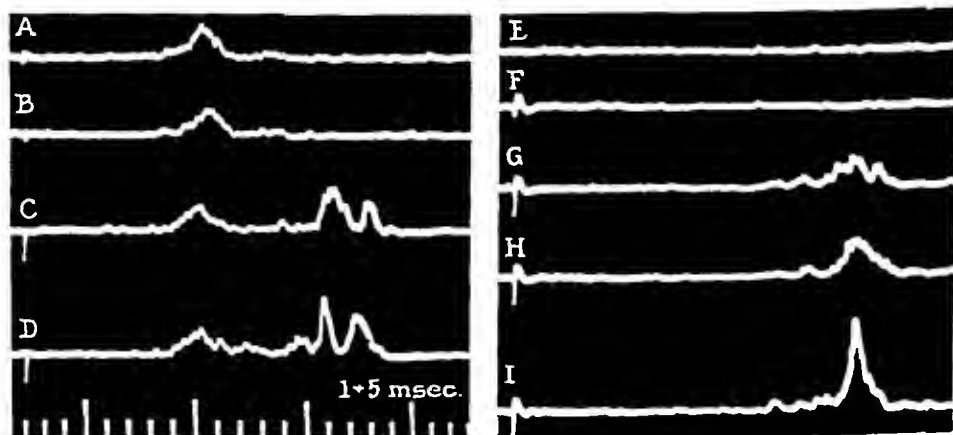


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nerve as the result of stimulating the sural nerve (A to D) and the gastrocnemius nerve (F to I). Record E shows the electrical base line in the absence of specific stimulation. The strength of stimulation will be referred to as weak (group II) or strong (group III).

Weak stimulation of the sural nerve (7A, B) results in a reflex discharge into the peroneal nerve with a total latency approximating 6 msec. With strong stimulation, this discharge grouping is present as before, but added thereto is another discharge grouping (7C, D). The latency of the second discharge (group III reflex) cannot be estimated with certainty, but approximates 11 msec. The situation is only slightly different when the gastrocnemius nerve is employed for afferent stimulation, for weak stimulation does not always result in any reflex discharge into the peroneal nerve (Fig. 7F). On strong stimulation of the gastrocnemius nerves, group III reflexes regularly appear in the peroneal nerve (7G, H, I) with the same time relationship as they exhibit on sural nerve stimulation (compare 7G, H, I with 7C, D). It will be remembered that flexor reflexes resulting from stimulation of the gastrocnemius nerves has been described by Sherrington (43) and Eccles and Sherrington (9). Presumably those reflexes frequently belonged to group III of the present classification. The not infrequent absence of group II reflex discharge on stimulating the gastrocnemius nerves is related to the poverty of medium sized afferent fibers (8, 29), but certainly there are sufficient to develop a subliminal field of excitation among the central neuron pools, and upon occasion to provoke a reflex discharge (cf. 21, Fig. 5A).

When the tibial nerve (less its branches to the gastrocnemius muscle) is substituted for the peroneal nerve to serve as an efferent reflex limb, little or no discharge attends stimulation of either sural or gastrocnemius nerves. Typically there is no reflex pathway from the gastrocnemius nerves to the (remainder of the) tibial nerve (Fig. 4H, I, Fig. 8B, C). A slight discharge may be found in the tibial nerve when stimulating the sural nerve, it is not increased apparently by strong stimulation (compare Fig. 8D, E). This last discharge is difficult to interpret, it might represent 'residual ipsilateral extension' or specialized reflex activity directed to the small muscles of the foot through the plantar divisions of the tibial nerve. Since the residual ipsilateral extension reflex is usually a rebound following preliminary inhibition (4, p. 81, Fig. 43), this seems at the moment an unlikely explanation, for the latency would then be much greater.

Since the experiments of Fig. 7 and 8 are performed with the dorsal and ventral root systems of the spinal cord intact, antidromic volleys in the motoneurons ensue whenever a muscle nerve is utilized for 'afferent' stimulation. The untoward effects of the antidromic volleys probably need not be considered when the antidromically activated motoneurons and the reflexly tested motoneurons belong one to the peroneal nucleus, the other to the tibial nucleus (32), but as between the divisions of the tibial nerve care must be exercised in forming conclusions. It has been shown above that the



antidromic volley would not seriously impede two-neuron-arc discharges coursing simultaneously through neighboring motoneurons, but this relative immunity need not extend to the group II and group III reflexes, for with their greater central latency they might find the motoneurons well advanced in the course of depression brought on by the antidromic volley in the neighboring motoneurons. The fact that little reflex effect is secured in the tibial nerve on stimulating the purely afferent sural nerve is a partial control, for in this case no antidromic volley is evoked. Furthermore, the number of group II fibers in the gastrocnemius nerves is small, making it unlikely that a substantial group II reflex would be found in the tibial nerve if a way were devised to avoid the antidromic volley. Finally the result of stimulation of cutaneous nerves on extensor muscles (*e.g.*, the vasti, crureus, semimem-

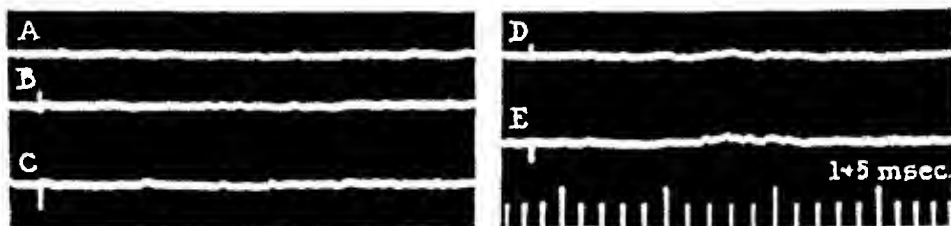


FIG 8 A, B, C—records obtained from the tibial nerve (less its constituent branches to the gastrocnemius muscle) A—blank sweep B, C—group II and group III strength stimulation respectively D, E—stimulation of the sural nerve at group II and group III strength respectively Note slight discharge in D and E

branosus, anterior part of the biceps femoris, soleus, gastrocnemius) is inhibition (43) rather than excitation (*cf.* also Fig 12A, B, C). There is reason to believe, however, that a group II or group III reflex would be realized among extensor motor nerves if the extensor inhibitory component of the flexor response evoked by stimulation of the plantar nerves were obviated by the use of natural stimulation (*cf.* discussion on the extensor thrust reflex in connection with Table 2).

*The shortest reflex pathway from one peripheral nerve to another* The most powerful reflex discharges transmitted from one hind limb nerve to another are those to be recorded in the peroneal nerve, or a suitable branch thereof, following stimulation of the tibial nerve. Because of this fact the tibial nerve to peroneal nerve reflex has been chosen as the system in which to examine the simplest reflex link from one nerve to another. The simplest link from one nerve to itself is the two-neuron-arc pathway. In order to establish a time reference by which to gauge the minimum central delay of the tibial nerve to peroneal nerve reflex, the group I reflex from the tibial nerve back into itself has been examined. Stimulation and recording leads are arranged so that the conduction distance for the two reflexes is comparable. Figure 9 illustrates an experiment performed after this manner. In observations A and G of Fig 9 are shown the reflex into the tibial nerve on stimulation of that nerve (compare with Fig 3). The latency of the two-neuron-arc reflex is

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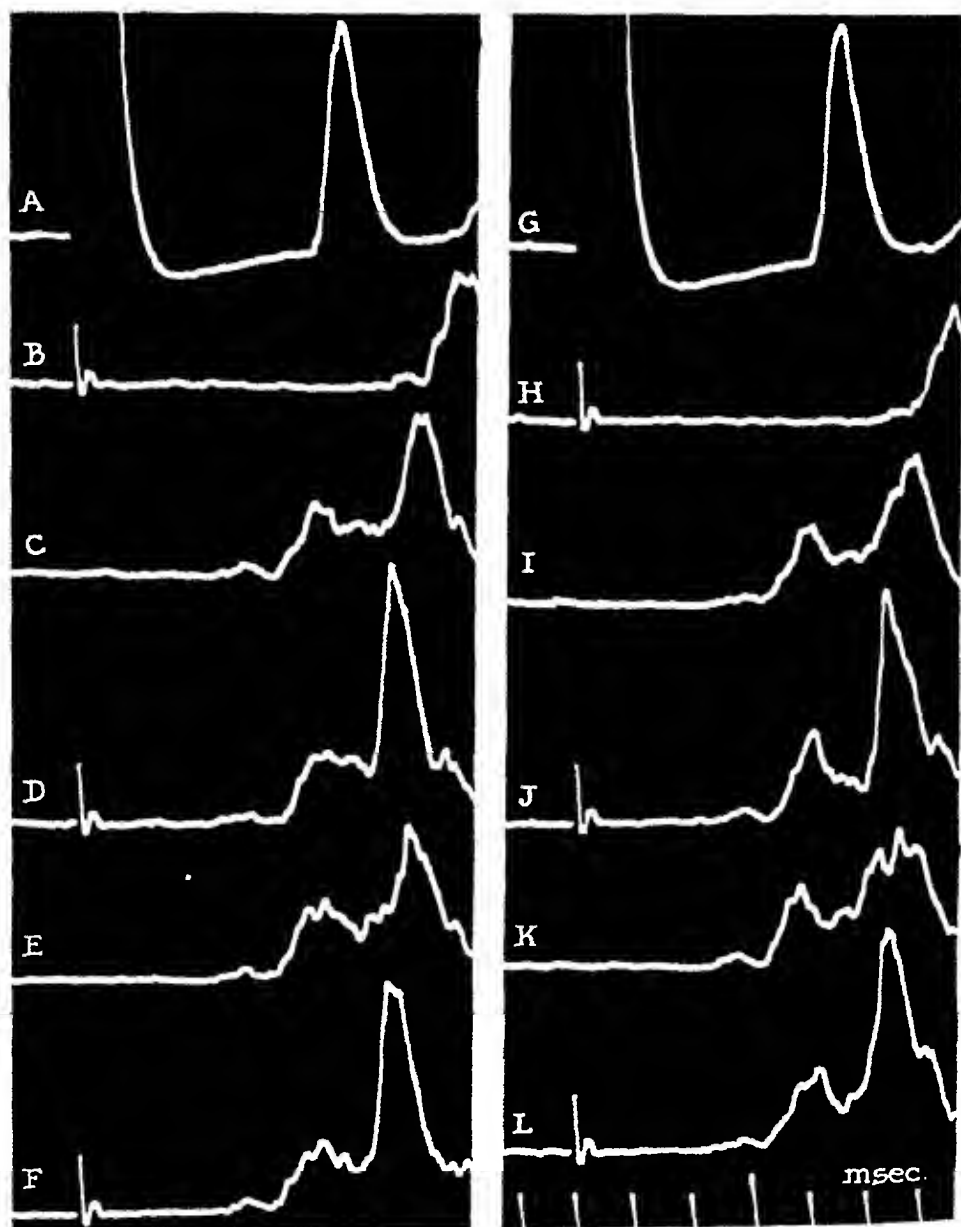


FIG 9 Reflex discharges on stimulation of the tibial nerve. A, G—the group I reflex recorded on the tibial nerve as in Fig 3. B, H—onset of group II reflex recorded on the peroneal nerve. In D, F, J, L the reflex into the peroneal nerve is facilitated by an antecedent shock to the tibial nerve, the reflex response to which is seen in C, E, I, K. Comparison of the latency of the facilitated reflex in the peroneal nerve with that of the reflex in the tibial nerve indicates that the minimum central pathway between the tibial nerve and the peroneal nerve contains an interneuron in series.

approximately 3.8 msec. Observations B and H illustrate the onset of reflex discharge into the peroneal nerve on stimulation of the tibial nerve. The latency of the first action is approximately 5.3 msec, i.e., 1.5 msec longer than that to be expected on transmission through two-neuron-arc pathways. It is obvious that the reflex discharge resulting from a single stimulation need not necessarily be transmitted through the shortest available pathway (cf. 10). Accordingly the discharge through the tibial nerve to peroneal nerve pathway has been examined under conditions of repeated stimulation calculated to yield the greatest facilitation of the response to the second of two successive shocks. A stimulation interval of 3.0 msec was found to be most effective. In Fig. 9C, -F and I-L, the responses to single and double stimulation are presented in alternation, the single responses being those to the first of the two shocks to the tibial nerve. By this plan one may judge the approximate electrical base line upon which is written the facilitated responses to the second shock. A prominent peak appears in records D, F, J, L with a latency of 4.9 msec as measured from the time of the second shock. Comparison of the facilitated responses D, F, J, L with the control responses B and H shows that the latency of the reflex into the peroneal nerve is reduced by 0.4 msec due to the action of the antecedent stimulation. On the other hand comparison of records D, F, J, L with records A and G shows that the latency of the reflex into the peroneal nerve, in spite of powerful facilitation, is 1.1 msec longer than that of the two-neuron-arc reflex back into the tibial nerve.

The latency differential between the two reflexes under consideration is not all referable to difference in central latency, for the reflex in the tibial nerve results from stimulation of group I afferent fibers, while the reflex in the peroneal nerve results from stimulation of group II afferent fibers. The proper correction for the differential afferent conduction under the conditions of the experiment illustrated in Fig. 9 amounts to 0.5 msec, or a little less (cf. also Fig. 5). Then, with allowance of 0.5 msec for the slower afferent conduction of the reflex into the peroneal nerve, there remains a latency differential of 0.6 msec between the two reflexes, all of which is attributable to excess central latency. Since this value is appropriate for the delay occasioned by a single synaptic relay (27), it appears that the minimum reflex pathway from the tibial nerve to the peroneal nerve contains one more neuron in series than does the reflex pathway back into the tibial nerve, and is, therefore, a three-neuron-arc pathway. Since the reflex pathway from the tibial nerve to the peroneal nerve contains all the paths pertaining to the classical reflex of the ankle flexor as studied by Eccles and Sherrington (10), it follows that the minimum pathway for this flexor reflex proper is one of three neurons.

*Group I and group II reflex discharges into flexor nerves.* Although the shortest pathway mediating the flexor reflex proper is one of three neurons (Fig. 9), the motoneurons of flexor muscles are supplied directly by primary afferent fibers and under the appropriate experimental conditions, two-

leads on the nerve to the tibialis anterior muscle approximates 38 cm, or 7 cm longer than the total reflex pathway obtaining in the experiment illustrated in Fig 3 The additional latency (0.6 msec) of reflex 10D, H over that of reflex 3A is only sufficient to account for conduction through the additional 7 cm length of pathway at a velocity of 117 M/sec Again, the records 10D, H were obtained by the use of shocks submaximal for the group I fibers of the peroneal nerve This is shown by the fact that the group I reflex is decreased on increasing the strength of stimulation, due to the increase in the antidromic volley, and consequent extension of central refractoriness (compare H with I in Fig 10) Also, with the increase in strength of the peroneal nerve shock, group II reflex discharges appear in the nerve to the tibialis anterior muscle

The peroneal nerve is a mixed nerve The low threshold reflex appearing in the nerve to the tibialis anterior muscle on stimulation of the peroneal nerve does so by virtue of the stimulation of afferent fibers arising in the tibialis anterior muscle, for, stimulation of the peroneal nerve after segregation of the tibialis anterior nerve results only in delayed discharges into the latter (*cf* Table 1) Conversely the late discharges seen in record I of Fig 10 must be due largely, but not exclusively, to the stimulation of cutaneous afferent fibers reaching the peroneal nerve trunk through its superficial branch Thus the initial discharge into the tibialis anterior muscle, on stimulating the parent peroneal trunk has the latency, threshold, and distribution features to be expected of a group I reflex In this connection it will be noted that Sherrington (38), Asayama (1) and Denny-Brown (5) have described the tendon-jerk, or 'pluck' reflex in flexor muscles, and that Forbes Campbell and Williams (11) and Matthews (28) have demonstrated afferent responses to stretch of flexor muscles Since the two-neuron-arc reflex does not appear in the pathway of the flexor reflex, there is ample reason to regard the two-neuron-arc connections of flexor muscles as devoted to the mediation of the tendon-jerk or 'pluck' reflex exhibited by those muscles

In connection with Fig 9 and 10 it has been seen that the latency of the group II reflex discharges is shortened by antecedent stimulation, as would be expected from the experiments of Eccles and Sherrington (10) In the absence of antecedent stimulation discharges through the three-neuron-arc pathway of the group II flexor reflex are almost (Fig 9B, H) or quite absent (Fig 4M, 7, 10) *i.e.*, subliminal, when the afferent stimulation is applied to a peripheral nerve, even though this stimulation be powerful But, just as flexor reflex activity is shunted into the shorter available paths by facilitation (Fig 9, 10), so is it similarly advanced by shifting the site of afferent stimulation from a peripheral nerve to a dorsal root

Figure 11 presents an experiment in which are compared the reflex discharges into the deep peroneal nerve by stimulation of the tibial nerve, the S1 dorsal root and the L7 dorsal root The deep peroneal nerve supplies the tibialis anterior, extensor longus digitorum and extensor brevis digitorum, all three of which respond in the great flexion reflex (43) It is a convenient nerve

neuron-arc reflex discharges may be demonstrated in flexor nerves. The experimental conditions are those by which similar discharges may be recorded in the extensor paths (Fig 1, 3, 9)

Figure 10 illustrates the reflex discharges to be found in the nerve to the tibialis anterior muscle on stimulation of the tibial nerve and the peroneal

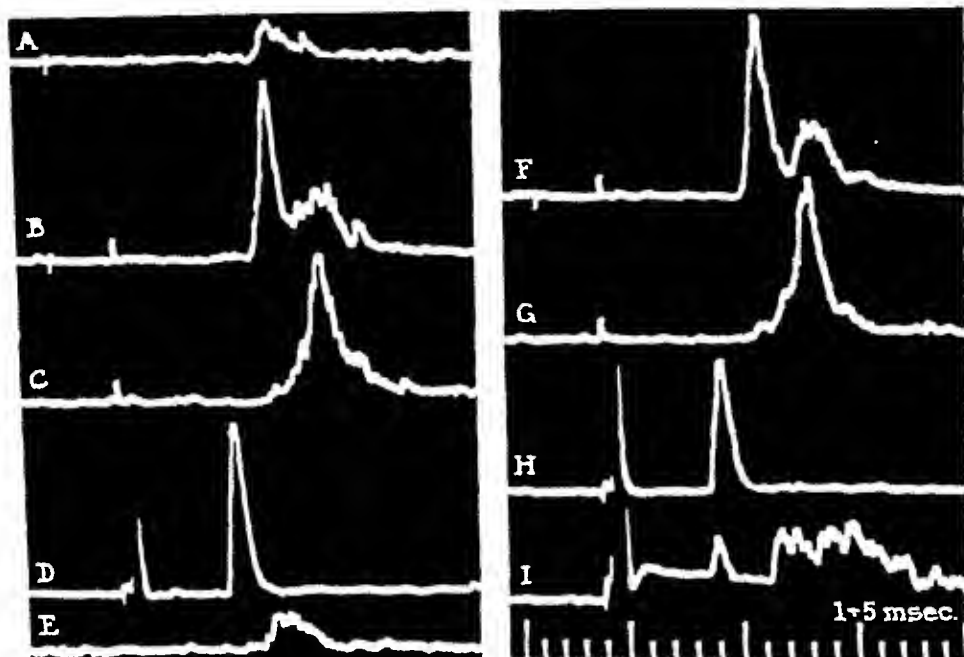


FIG 10 Reflex discharges into the nerve to the tibialis anterior muscle (ankle flexor). A, E—submaximal group II reflex evoked by stimulation of tibial nerve. C, G—maximal group II reflex evoked by stimulation of the tibial nerve. B, F—facilitated reflex by combined stimulation of the tibial nerve. D, H—group I reflex evoked by stimulation of the parent trunk of the peroneal nerve at approximately half maximal group I strength. I—when the peroneal nerve shock is increased the group I reflex volley is diminished by the consequently greater antidromic volley and group II (and group III) reflex discharges appear *de novo*.

nerve (the latter being the parent trunk for the nerve to the tibialis anterior muscle). Observations A, C, E, G show the reflex discharges resulting from single shock stimulation of the tibial nerve, the shock being stronger for C and G than for A and E. Observations B and F present the result of combined stimulation of the tibial nerve, the stronger shock preceded at an interval of approximately 30 msec by the weaker shock. Comparison of B and F with C and G shows that the latency of the response to the second tibial nerve shock is shortened by 0.8 msec to a value of 5.8 msec. In contrast, stimulation of the parent trunk of the peroneal nerve results in a group I (two-neuron-arc) reflex into the nerve to the tibialis anterior muscle (Fig 10D, H). The latency for this group I reflex is 4.6 msec. The reflex pathway from the stimulating electrodes through the spinal cord and back to the recording

and S1 ventral root was proved by the recording of directly conducted alpha spike potentials between the nerve and the roots. Disregarding the two-neuron-arc discharge in C and D for the moment, for this represents reflex activity arising from the deep peroneal nerve rather than the tibial nerve, the latency of the next discharge in order is 4.4–4.6 msec, encompassing some slight variation from one observation to another. On advancing the site of stimulation from the tibial nerve to the dorsal root, therefore, the latency of the flexor reflex proper is reduced from 7 msec to approximately 4.5 msec. Some fraction of this latency differential is referable to the shortening of the afferent limb of the reflex. Since the tibial nerve contains group I fibers in addition to the group II fibers mediating the reflex in question, the exact allowance for afferent conduction cannot be measured. Assuming that as much as 1.7 msec afferent conduction time is involved (this would represent 12 cm conduction at 70 M/sec) there is still a shortening of central latency amounting to 0.8 msec, which occurs by virtue of 'skipping a synapse' (26).

The most prominent discharge peak in C and D of Fig. 11 has a latency of 5.6 msec, which is shorter than that of the initial discharge in A and B only by the equivalent time for conduction from the tibial nerve to the S1 dorsal root. It would seem that this peak in C and D represents the same 'order' of reflex discharge as the initial discharge in A and B, but it has gained greatly in potency.

The effect of advancing the site of stimulation from the peripheral nerve to the dorsal root, then, is an intensification of the discharge through the shorter available paths at the expense of discharge through the longer paths, in addition to the simple shortening of latency due to shortening the afferent limb of the reflex pathway. It seems probable that a decrease in central latency brought about in this manner accounts for the apparent long afferent conduction time and correspondingly short minimum central reflex time calculated for the flexor reflex by Eccles and Sherrington (10), for they measured the afferent time by the difference in reflex latency when stimulating the tibial (popliteal) nerve and the S1 (8th post-thoracic) dorsal root.

*On the conditioning of two-neuron-arc reflexes.* According to the evidence of the present experiments, the segmental reflex discharge is constituted of two-neuron-arc discharge directed in varying ratio, depending upon the segment employed and other considerations, to muscles of flexor and extensor action, together with multineuron-arc discharges almost exclusively directed to muscles of physiological flexion, irrespective of the segment employed. Some of the vagaries of conditioning experiments in which segmental two-neuron-arc reflex discharges are employed as test volleys are undoubtedly due to the dual nature of those discharges. It is frequently found, on causing a two-neuron-arc volley to fall during the multineuron-arc discharge in response to an antecedent shock, that the two-neuron-arc volley is subjected simultaneously to temporal facilitation and spatial inhibition. The explanation for this anomalous behavior becomes clear when the components of the

structure for the recording of flexor activity. As a preface to the consideration of Fig. 11, one should bear in mind the differences between the L7 and S1 spinal segments in relation to the three muscles served through the deep peroneal nerve. The L7 segment regularly supplies the muscles mentioned (35, 10). The S1 segment may contribute a small twig to the peroneal nerve when the arrangement of the plexus is of the postfixed type. One would ex-

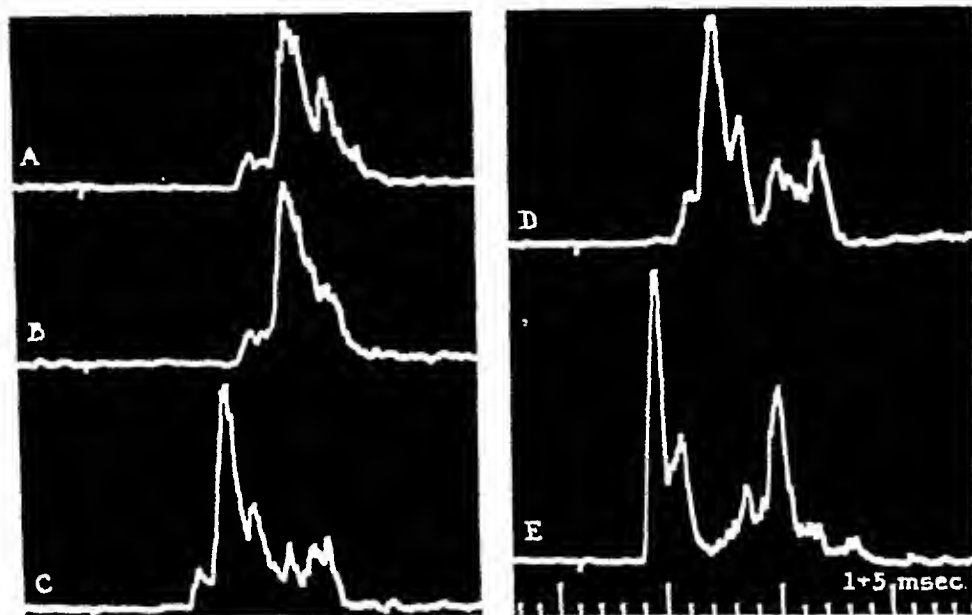


FIG. 11 Reflex discharges into the deep peroneal nerve (supplying MM. tibialis anterior, extensor digitorum longus et brevis digitorum). A, B—reflex discharge on stimulation of the tibial nerve, C, D—reflex discharges on stimulation of the S1 dorsal root. E—reflex discharge on stimulation of the L7 dorsal root. Note 'synapse skipping' and concentration of activity through shorter reflex chains when stimulation is advanced from peripheral nerve to dorsal root, also threshold two-neuron-arc discharge when stimulating S1 dorsal root compared with powerful two-neuron-arc discharge when stimulating L7 dorsal root.

pect to find, therefore, little (postfixed preparation) or no (prefixed preparation) two-neuron-arc reflex discharge from the S1 dorsal root to the deep peroneal nerve, but a not inconsiderable two-neuron-arc discharge from the L7 dorsal root to the deep peroneal nerve.

Figure 11A and B illustrates the reflex discharge recorded from the deep peroneal nerve on stimulating the tibial nerve. The latency of this discharge is approximately 7 msec. Observations C and D illustrate the reflex discharge similarly recorded, on stimulating the S1 dorsal root. As might be expected there is a small, but quite regular two-neuron-arc discharge appearing after a latency of 3.2 msec. (compare C and D with E, obtained by stimulation of the L7 dorsal root, and which contains a powerful two-neuron-arc discharge). The arrangement of the plexus in this preparation was postfixed, and direct fiber connection between the deep peroneal nerve and both the S1 dorsal root



Similar results can be obtained when stimulating dorsal roots while segregating the extensor and flexor components on the motor side (*cf* also 33), but the method here illustrated seems preferable in theory for the input to the spinal cord is restricted so that only the flexor or the extensor testing two-neuron-arc discharges are elicited, depending upon choice. On the contrary, when segregation is effected on the motor side, stimulation of dorsal roots will cause activation of both flexor and extensor two-neuron-arcs in parallel, and although only one or the other is recorded, the way is open for undesirable interaction, and the possibility of confusion phenomena within the testing system remains.

*The classification of ipsilateral hind limb reflexes* Throughout most of the present paper the reflex discharges described have been classified according to the arbitrary groups outlined in the introduction, but certain obvious correlations with hind limb reflexes as they are known on the basis of motor performance have emerged. Table 2 forms a summary of these correlations and of the foregoing experiments.

Table 2 *The classification of ipsilateral hind limb reflexes*

Group	Approximate afferent fiber range	Central connections	Origin	Destination	Type of reflex
I	20-12 $\mu$	Direct, or two-neuron-arc reflexes	(a) extensor muscles	The extensor muscle from which it arises	Myotatic reflex (the tendon-jerk)
			(b) flexor muscles	The flexor muscle from which it arises	Flexor tendon-jerk (the pluck reflex)
II	12-6 $\mu$	Multineuron-arc reflexes Three-neuron-arc minimum	(a) skin	flexor muscles	The flexion reflex
				extensor muscles (slight)	Residual ipsilateral extension?
III	6-1 $\mu$	Multineuron-arc reflexes ? minimum	(b) muscle (slight)	flexor muscles	The flexion reflex
			(a) skin	flexor muscles	'Delta' flexion reflex
IV	unmyelinated (C) fibers		(b) muscle	flexor muscles	'Delta' flexion reflex
					( <i>cf</i> 3, 2)

segmental reflex are segregated as in the experiment presented in Fig 12. The extensor two-neuron-arc component is obtained by stimulating the nerves to the gastrocnemius muscle, the flexor two-neuron-arc component by stimulating the deep peroneal nerve, and the multineuron-arc component by stimulating the sural nerve. Records are obtained from the L7 or S1 ventral root.

Record A of Fig 12 shows the extensor two-neuron-arc reflex recorded from the S1 ventral root following stimulation of the gastrocnemius nerves

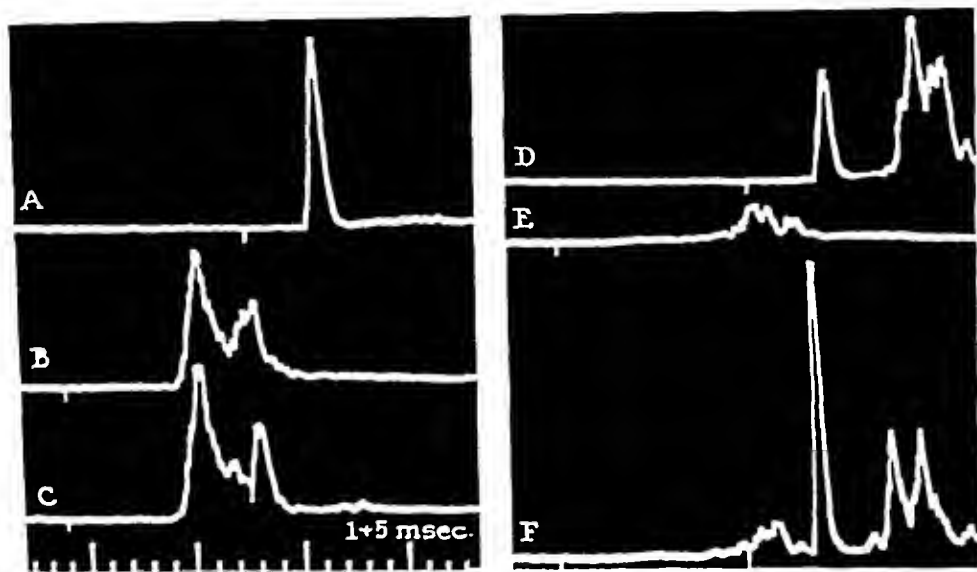


FIG 12 Conditioning of extensor two-neuron-arc and flexor two-neuron-arc reflexes by stimulation of a cutaneous nerve (sural). A, B, C recorded from the S1 ventral root. A—gastroc nerves stimulated. B—sural nerve stimulated. C—combined stimulation of sural and gastroc nerve at interval of 8.5 msec. The gastroc two-neuron-arc is inhibited, D, E, F—recorded from the L7 ventral root. D—deep peroneal nerve stimulated—note two-neuron-arc and later discharges. E—sural nerve stimulated. F—combined stimulation of sural nerve and deep peroneal nerve. The two-neuron-arc reflex is facilitated both spatially and temporally by the action of the sural nerve volley.

Record B shows the multineuron-arc discharge resulting from stimulation of the sural nerve. On combining these two stimulations (record C) at an interval of 8.5 msec the extensor two-neuron-arc reflex is inhibited, although few if any extensor motoneurons could have discharged in the earlier reflex. Record D illustrates the response recorded from the L7 ventral root on stimulation of the deep peroneal nerve. The two-neuron-arc discharge is here followed after an interval by multineuron-arc discharges. Record E shows the response evoked by stimulation of the sural nerve. In record F, sural and deep peroneal nerve stimulation are combined as were the sural and gastrocnemius nerve stimulations in 12C. In this case, however, the flexor two-neuron-arc discharge is facilitated, both temporally and spatially.

that through flexor two-neuron-arcs facilitated by the transmission of multi-neuron-arc reflex action

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It will be seen at once that the outstanding omission from Table 2 is the ipsilateral reflex of extension known as the extensor thrust (40). This reflex is elicited by pressure or 'deep touch' on the planta (37, 39), whereas nocuous stimulation of the same area elicits flexion of the limb. The plantar nerves are essential to the transmission of the extensor thrust reflex (39), and hence mediate the afferent limb of this reflex. Yet stimulation of these nerves themselves yields flexion (42), accompanied by relaxation of extensors. The extensor reflex is presumably inhibited by the concomitant and prepotent flexor reflex. The extensor thrust seems, therefore, not to be a group I reflex, for with simultaneous combination a group I reflex would outstrip the flexion reflex and thereby escape inhibition. One would expect the extensor thrust to belong in group II. Undoubtedly more prominent group II discharges to extensor muscles would be found in the fore limb, in which the stimulation of digital nerves promotes ipsilateral extension (6). Considerations such as these invoke again the problem to which there is still no decisive answer: it is not yet known to what extent fractionation of spinal centers is determined by anatomical limitation of neuron connections, or to what extent it is attained primarily by functional means, with direct inhibition possibly playing a dominant role.

#### SUMMARY

The reflex function within the hind limb of myelinated afferent fibers has been examined. Three sub-groups of these fibers are recognizable. The large fibers form direct connections with the motoneurons, the medium and small fibers connect with interneurons.

Reflex discharge mediated through the direct (two-neuron-arc) connections reflects only into the muscle, head of a muscle, or combination of muscles, the large afferent fibers of which are subjected to stimulation. Because of the identity of distribution holding for the two-neuron-arc discharge and the myotatic reflex, it is concluded that the two-neuron-arc pathways are reserved for mediation of the myotatic reflex.

Multineuron-arc discharges, evoked by stimulation of medium and small afferent fibers, are directed for the most part into the nerves of flexor muscles, and represent the flexor reflex proper. The minimum central pathway devoted to this reflex is one of three neurons.

Under appropriate conditions the flexor muscles receive excitation through arcs of two neurons as well as through the multineuron reflex arcs. The conditions are exactly those governing the transmission of two-neuron-arc excitation to extensor muscles. It is concluded that the flexor two-neuron-arc reflex represents the flexor tendon-jerk, or 'pluck' reflex in contradistinction to the flexor reflex proper.

The segmental reflex discharge recorded from a ventral root on stimulation of the dorsal root of the same segment contains three major elements, an extensor two-neuron-arc, a flexor two-neuron-arc and flexor multineuron-arc discharges. Reflex activity through extensor two-neuron-arcs is inhibited,



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of stretch-movement. The time relations of the movement were recorded by the use of a photoelectric cell. Although the mechanism is not as flexible as one might desire, it behaved in a constant manner for any given setting of the controls, and served the immediate purpose of producing a brief stretch synchronized with the sweep of the cathode ray oscillograph.

The gastrocnemius muscle was employed for all the present observations. The tendon together with its insertion into the tuberosity of the calcaneus was fully isolated and freed from the insertion of the soleus muscle. Extensive denervation of the limb was regularly practised. Since the ventral roots were cut for the purpose of recording the reflex responses, the muscle itself was unable to participate in the reflex response. This arrangement is particularly useful, for the jerk reflex proper is divorced from all secondary phenomena such as the response to active tension, the myotatic appendage and incipient clonus.

Figure 1 illustrates stretch-evoked afferent discharges recorded from the nerve to the medial head of the gastrocnemius muscle. Records A, C, E show, by photoelectric recording, the extent and duration of the stretch-movement imparted to the gastrocnemius tendon to provoke the afferent discharges recorded in B, D, F respectively. The afferent discharge 1B is caused by a stretch of 1 mm effected in 40 msec. It will be seen that the recognizable

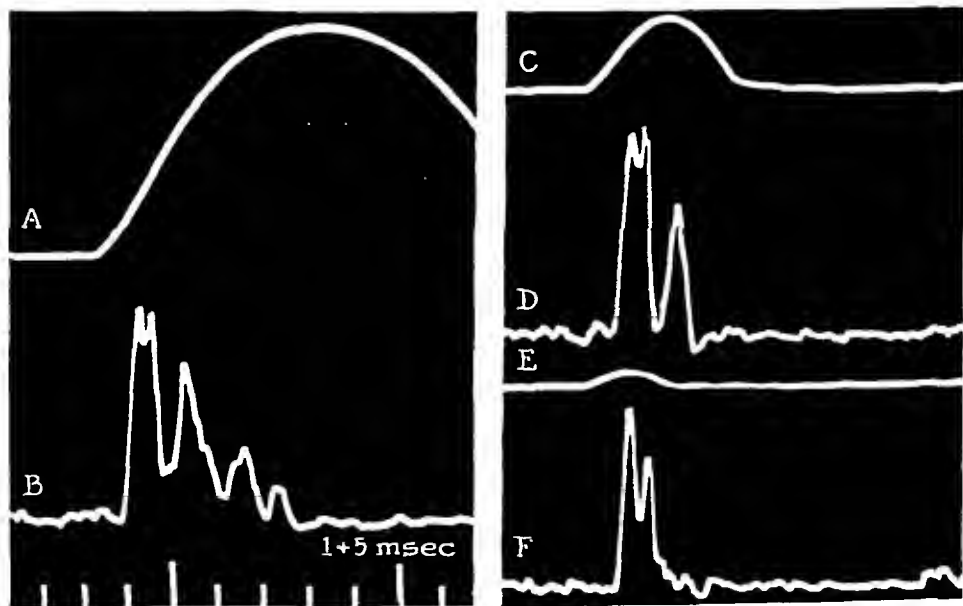


FIG 1 Afferent responses to brief stretch of M gastrocnemius recorded from the nerve to the medial head of that muscle. In A, C, E are recorded the extent and duration of the stretches employed to obtain the responses B, D, F respectively. The extent of stretch indicated in A is 1 mm, in C, 0.3 mm, in E, 0.05 mm. Most of the observations illustrated in this paper were obtained by the use of 0.2–0.3 mm stretches. Time in 1 and 5 msec intervals.

# CONDUCTION AND SYNAPTIC TRANSMISSION OF THE REFLEX RESPONSE TO STRETCH IN SPINAL CATS

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IN THE PRECEDING PAPER (14) it was found that the reflex discharge provoked by stimulation of the large ( $20\text{--}12\mu$ ), low-threshold muscle afferent fibers, and transmitted through arcs of two neurons (12) possesses the characteristic and restricted distribution of the myotatic reflex (9). The identity of distribution was so rigidly maintained that it seemed justifiable to attribute to the two-neuron-arc pathways the mediation of myotatic reflexes. The concept, of course, is not new (8), but the older evidence in support of the concept lost weight with the advent of more precise measurements of the delay involved in synaptic transmission. Furthermore, one is constantly aware of the danger inherent in drawing conclusions as to natural reflex performance from experiments utilizing electrical stimulation of bare nerve trunks. Therefore, the conclusion derived from such experiments could be sustained and accepted only if the myotatic reflex evoked by the appropriate natural stimulus, viz, stretch, were found, on reinvestigation, to have the same characteristics of conduction and synaptic transmission as the two-neuron-arc reflex discharge obtained by stimulation of muscle nerves. The present experiments were designed to examine the temporal course of the reflex response to phasic stretch. Some of the observations were mentioned briefly in a preliminary note (13).

The experiments were performed on cats made spinal by transection accomplished through the atlanto-occipital membrane. Following transection artificial respiration was instituted and the ether anaesthetic discontinued. The pelvis was fixed by heavy pins, the femur and tibia by drills. These were held by heavy standards which were firmly attached to the operating platform, of two-inch oak, supported by three-inch I-beams on a sand-filled oak table. Satisfactory fixation of the muscle origins was thus obtained. The stretching mechanism was similarly mounted on another sand-filled table. The use of two such tables separated by an air gap prevented the direct transmission of mechanical jar to the preparation. A hooked steel wire impaling the tendon of the gastrocnemius muscle served to connect it to the stretch mechanism, which consisted of a plunger operated by two solenoids arranged in opposition to each other. The solenoids were activated by condenser discharges timed by the usual stimulating circuit. One solenoid was arranged to pull, through the steel wire, on the freed tendon of the muscle. The other served to check the first so that the extent and duration of the stretch could be limited. In order to overcome inertia, the solenoid plunger was allowed to gain its momentum before acting upon the wire affixed to the tendon. A break contact on this wire fed an impulse to the amplifier to signal the onset



bell and Williams (4), although Jolly's earlier measurements indicated the existence of a true end-organ delay (7)

Figure 2 presents an experiment designed to measure the conduction velocity of the afferent fibers mediating the response to stretch. The afferent discharge was first recorded from the first sacral (S1) dorsal root, severed from its connection with the spinal cord, and subsequently from the tibial

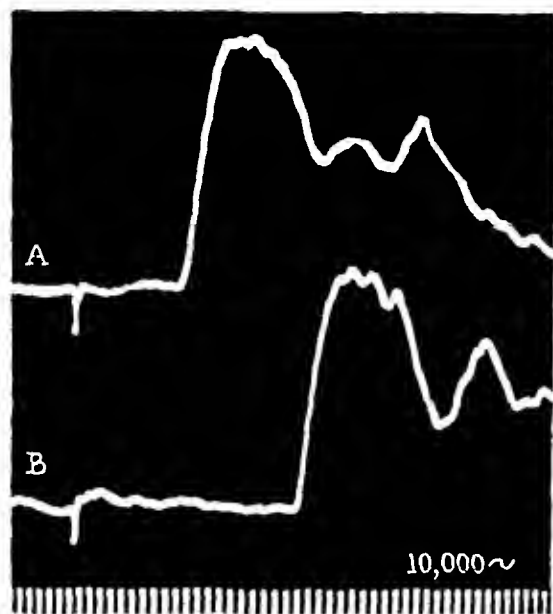


FIG 2 Conduction of stretch-evoked afferent response. A—afferent response recorded from tibial nerve, B—from S1 dorsal root. Conduction distance—12 cm. Differential latency—1.025 msec. Conduction rate—117 M per sec. Time—10,000 cycles.

nerve in the popliteal space. The conduction distance between the proximal (to the muscle) recording leads of the two pairs was 12 cm. The difference in latency of conduction is 1.025 msec, which yields a conduction rate of 117 M per sec.

In the graph of Fig. 3 are plotted the results of five experiments such as that illustrated in Fig. 2. Each point on the graph represents an experiment, and relates the latency difference between the discharge recorded at two points along the afferent pathway to the conduction length of the pathway between the two recording stations. The observations at short conduction distances were made with both recording stations on the tibial nerve, or on one or both of the nerves to the gastrocnemius muscle.

Those observations at long conduction distances were made with one of the electrode pairs on the dorsal root. In two experiments the conduction velocity of a volley evoked by electrical stimulation of the intramuscular branches of the nerve to the medial head of the gastrocnemius muscle was determined in addition. All of the observations from the various preparations fall about a straight-line plot having a slope of 116 M per sec. It follows that fibers mediating the afferent response to stretch fall among those of highest velocity in the muscle nerve. Within the limits of measurement the secondary peaks of the afferent discharge evoked by stretch represent activity in fibers of similar properties (*cf* Fig. 2). Thus, one criterion for the identification of the myotatic reflex pathway with the two-neuron-arc pathway is satisfied.

Comparison of the afferent discharges encountered in the present experi-

discharge occurs during the period of stretch-movement, and takes the form of a succession of imperfectly synchronized volleys. When the stretch is reduced to approximately 0.3 mm effected in 1.7 msec, the afferent discharge, 1D, is correspondingly reduced in duration. The last two discharge peaks present in 1B are no longer realized, and the second peak is reduced in amplitude. The initial discharge peak, however, is intact. Further reduction of the stretch to approximately 0.05 mm causes a further reduction in the afferent response, 1F, only the initial discharge remaining, this is decreased and further 'splintered'. The degree of stretch employed for response 1F is still well above threshold. It will be remembered that Denny-Brown and Liddell (3) obtained a jerk reflex in the decerebrate supraspinatus muscle with a stretch of approximately  $8\mu$ .

In the records illustrated in Fig. 1 there is a degree of diphasicity which results in emphasis of the relatively synchronous discharges of stretch-movement over the asynchronous activity apparently maintained throughout the period of muscle elongation. The latter discharges are seen to better advantage in Fig. 5B and 6B.

The latency of the afferent discharge as recorded in Fig. 1 approximates 0.7 msec, of which a considerable fraction is consumed in simple conduction. A number of attempts to obtain a valid estimate of 'end-organ delay' have been made. In a typical experiment the afferent response to stretch is first recorded. Then, with stimulating electrodes buried in the heart of the muscle belly, the preterminal nerve bundles are stimulated, the conducted action being recorded as before. The latency of the naturally evoked volley is longer than that of the electrically stimulated volley by as little as 0.2-0.3 msec. The electrically stimulated volley is conducted, of course, in both afferent and motor fibers, but this fact has no practical bearing on the outcome of the experiment for as will be seen in connection with Fig. 2 and 3, the afferent impulses provoked by sudden stretch travel in the forefront of the action potential of the mixed nerve. Values obtained by this method must be maximal rather than minimal values, for error due to possible spread of the stimulus or improper placement of the stimulating electrodes would decrease the apparent conduction time for the electrically stimulated volley, and by subtraction this would increase the apparent latency of the stretch response.

Some fraction of the 'excess latency' of the stretch-evoked response is needed for transmission of the tension wave from the free end of the tendon to the site of the first-responding receptors, wherever that may be. Another small fraction of time must be allocated for conduction from the point at which the end-organ excites the afferent fiber to the point at which the electrical stimulus excites the fiber. What little time is left probably may be accounted for in terms of a 'temps utile'. Altogether the evidence appears to militate against the view that a specific end-organ delay is involved in the stimulation by stretch of the particular end-organs mediating the initial afferent response. In essence, this is the conclusion reached by Forbes, Camp-

proximate the latency in 4C, which it does to within 0.1–0.2 msec. The slight additional latency of the naturally evoked reflex is referable to the greater dispersion of the afferent volley caused by long afferent conduction and natural stimulation. The initial reflex discharge in response to stretch, then, is transmitted through arcs of two neurons.

The initial volley of the stretch-evoked reflex is followed at an interval of approximately 0.8 msec by a second reflex volley, which is much larger than

the first. The second volley like the first is transmitted through arcs of two neurons. Figure 5A, B, C illustrate in order the time relations of stretch, the afferent response recorded from the gastrocnemius nerves, and the reflex response recorded from the S1 ventral root in another preparation. The latency of the initial reflex volley is approximately 3.9 msec, that of the second reflex volley 0.8 msec longer. Since there are two clearly defined successive afferent discharges impinging upon the spinal cord (5B), one would expect to realize two reflex volleys. Furthermore, the interval between the two reflex volleys is equal to or very slightly less than the interval between the two afferent volleys (compare 5B and 5C). This being so the central delay of the second reflex volley is of the same order of magnitude as that of the first reflex volley.

The latency differential between the two successive reflex volleys is not sufficiently long to admit the possibility that the second reflex volley is evoked through three-neuron-arc pathways by the action of the afferent impulses set up at the very beginning of stretch. The three-neuron-arc reflex discharge results from the stimulation not of group I fibers, but of group II

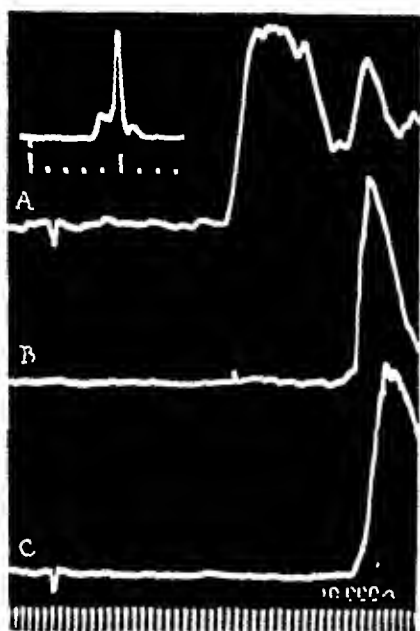


FIG. 4. Reflex latency of the response to brief stretch. A—afferent response recorded from the S1 dorsal root. B—segmental two-neuron-arc reflex (stimulus S1 dorsal root, recording S1 ventral root). C—reflex evoked by stretch. The sum of the latencies in A and B approximates that of C, showing that the initial reflex response to stretch is conducted through arcs of two neurons. Inset—the complete reflex response in this experiment consists of three successive volleys. Time for A, B, C—cycles. Time for inset in 1 and 5 msec intervals.

fibers (13). When the two groups of fibers are stimulated in the thigh, the group II volley reaches the spinal cord 0.4 msec after the group I volley, the conduction distance being 12 cm (14, Fig. 5). The pathway from the mid-belly of the gastrocnemius muscle to the spinal cord is 18–20 cm in length. Assuming that group II fibers were to participate in the first afferent

ments with those recorded by Matthews using single fiber preparations (15) suggests that the relatively synchronized volleys of stretch-movement have their origin in the A type receptors, by virtue of low threshold, rapid response and sudden cessation. This would indicate that the muscle spindles are the sensitive organs originating these discharges.

The succession of afferent discharge peaks may represent the successive recruitment of end-organs to the active 'pool,' or the repetitive discharge of end-organs recruited at the onset of stretch movement. In the latter case the rate of firing would approach 1000/sec, if the discharge peaks represent the rate, and not a multiple of the rate, of the individual end-organ discharges. This figure seems high, but the nerve fibers are capable of responding at such rates, for a short time at least (6). Employing the single fiber technique, the highest discharge rate observed by Matthews (15) was 500/sec during a stretch of 5 mm at the rate of 25 cm/sec. The highest rate of stretch attained in the present experiments was approximately 35 cm/sec over a shorter distance, so it is possible that a higher rate of firing was induced. Since the stretch-movement usually lasted 2-3 msec, some repetitive discharge certainly would have occurred, but successive recruitment is not thereby eliminated.

In order to examine the central latency of the reflex response to stretch the following procedure was adopted. The reflex was first recorded from the S1 ventral root. This appears in the inset of Fig. 4 and again in 4C. Only the initial volley is to be seen in Fig. 4C. The S1 dorsal root was then stimulated at a shock strength which yields a two-neuron-arc reflex discharge of approximately the same size as the initial stretch-evoked reflex volley. A record of the segmental two-neuron-arc reflex volley is found in 4B. The dorsal root was then severed at its junction with the spinal cord and equipped with recording leads. The proximal (to the muscle) recording lead was placed as close as possible to the point occupied by the cathode of the stimulating pair employed to obtain record 4B. With this disposition of leads the afferent response to the stretch was recorded, this is found in 4A. Now, if the initial volley of the stretch-evoked reflex is indeed transmitted through arcs of two neurons as predicted (14), the sum of the latencies in 4A and 4B should ap-

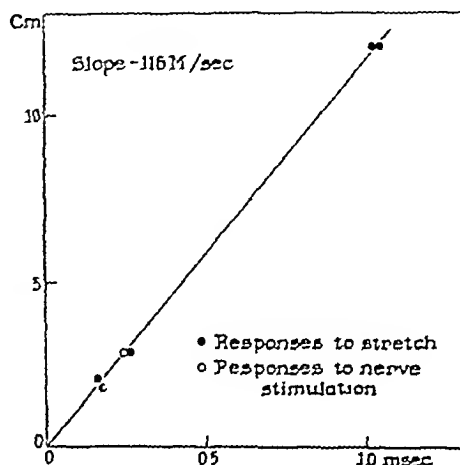


FIG. 3 Graph to show average conduction rate of afferent response to stretch in five experiments. Each point represents the difference in latency of the afferent response recorded at two points along the afferent pathway (as in Fig. 2) plotted against the conduction distance between the two points. For two of the experiments the conduction rate for an electrically stimulated volley is plotted. Average maximum conduction rate—116 M per sec.

an experiment in which this occurred Records A, B, C show in order the stretch imposed upon the gastrocnemius muscle, the afferent response recorded from the S1 dorsal root, and the reflex response recorded from the S1 ventral root Although the afferent response is dispersed over a period equal to that of the stretch, and contains two prominent discharge peaks, the reflex consists of a single volley, no more dispersed than is the response obtained by single shock stimulation of the gastrocnemius nerves (12, 14) The latency for this reflex is approximately 4.9 msec, which is almost identical to the latencies of the second reflex discharges in Fig 4 and 5 The time relationship between the second afferent discharge peak (6B) and the reflex (6C), moreover, accords with the conclusion that the second afferent volley is the immediate provocative agent for the reflex recorded

#### COMMENT

For the duration of stretch impulses are continually bombarding the motor nucleus at an intensity greater than that maintained during the 'resting posture' of the muscle Signalling the onset of stretch-movement, and for the duration of stretch-movement, regularly spaced synchronized afferent volleys appear, as though superimposed on a continuum of activity The first of these afferent volleys may (Fig 4, 5) or may not (Fig 6) provoke a discharge of motoneurons By the time the second afferent volley reaches the motor nucleus conditions have changed Depending upon the initial reflex discharge a few motoneurons may be refractory, but the greater part of the motor nucleus is more excitable than formerly by virtue of the continued impact of afferent activity Motoneurons are more readily available to the second afferent volley and a large reflex volley results The central latency of this volley may be even a little shorter than that of the initial reflex volley (temporal facilitation) Subsequent afferent impulses usually fail to reach motoneuron threshold, due possibly to the number of motoneurons already fired by the immediately preceding volleys The end result is a reflex discharge of shorter total duration than that of the afferent influx One must bear in mind the possibility that some of the afferent impulses may mediate direct inhibition (10) rather than excitation to the motor nucleus thus shutting off rather than aiding the reflex, or they may serve to feed ascending paths without concern for the local reflex mechanism Undoubtedly the reflex picture would be different if the stretched muscle were allowed to participate in the reflex action, as was necessarily the case in earlier work on the tendon jerk which depended largely upon either the action potential of the muscle or upon mechanical registration for a means of recording

The present experiments associate the large (group I) afferent fibers and two-neuron-arc connections with the mediation of the myotatic reflex It is not known whether or not the smaller afferent fibers in the gastrocnemius nerves partake in the afferent response to stretch of the degree imposed in the present experiments With greater extension of the muscle other than the

response to stretch, the resulting group II volley would reach the cord dorsum at least 0.6 msec after the group I volley. To this must be added another 0.6 msec for the additional synaptic relay of a three-neuron-arc, to give a minimum of 1.2 msec by which the second reflex volley should trail the initial reflex volley. This minimum value is considerably longer than the observed interval of 0.8 msec.

The discharges mediated through arcs of three neurons are directed into flexor channels (14). It is known from the experiments of Denny-Brown (2) that activity may appear in the tibialis anterior muscle as the result of sudden stretch of the gastrocnemius muscle, but it does so during the heart of the silent period of the extensor muscles. According to Denny-Brown the latency for activity in the tibialis anterior muscle averaged 22.7 msec compared with 8.6 msec for the latency of the tendon-jerk reflex in the gastrocnemius muscle itself. The second reflex discharge in the present experiments, and the third when it is present (inset of Fig. 4), is obviously part of the tendon-jerk reflex proper, rather than a contribution to flexor activity associated with the silent period of the extensor muscles. In addition to these considerations there is the fact that the gastrocnemius nerves contain relatively few group II fibers. Group II reflexes of any order are not regularly produced by stimulation of the gastrocnemius nerves (14). For these various reasons it is concluded that the second reflex volley in response to brief stretch, like the initial volley, is conducted through arcs of two neurons.

When the stretch produced by the pulling solenoid used in these experiments is unchecked by the opposing solenoid, it may happen that a third reflex volley, of small size, is discharged (*cf.* inset of Fig. 4). This reflex volley occurs later rather than earlier than the hypothetical three-neuron-arc time discussed above, but the other arguments advanced in favor of the view that the second reflex discharge pertains to arcs of two neurons hold also for the third reflex volley when this is in evidence.

The initial afferent volley of the stretch response may prove subliminal for the motoneurons, in which case the reflex volley provoked by the second afferent volley may constitute the whole reflex response. Figure 6 presents

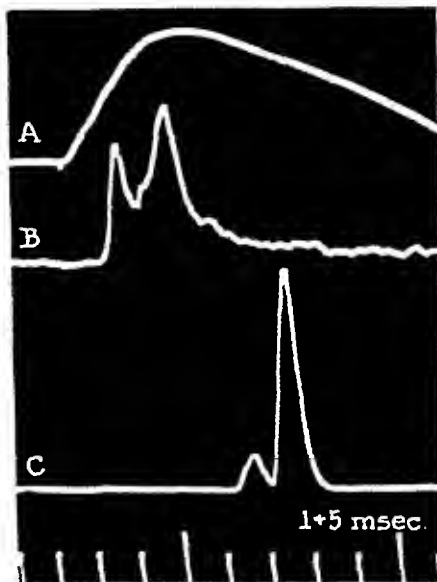


FIG. 5. Tendon-jerk reflex of *M. gastrocnemius*. A—stretch imposed upon the gastrocnemius muscle. B—afferent response recorded from the gastrocnemius nerves in the thigh. C—reflex response recorded from S1 ventral root. Time in 1 and 5 msec intervals.

## SUMMARY

The afferent response to brief stretch of the gastrocnemius muscle is mediated by large (group I) fibers at an average maximum velocity of 116 M per sec

There is little if any true delay at the sensient organs responding to stretch

The reflex response to brief stretch of the gastrocnemius muscle is transmitted through arcs of two neurons

It was previously shown (14) that the distribution of two-neuron-arc discharges accords with that of the myotatic reflex. For these several reasons it appears that the two-neuron-arc pathways are reserved for the mediation of myotatic reflexes

The calculated overall minimum latency for the tendon jerk reflex of the gastrocnemius muscle is approximately 5.95 msec

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large afferent fibers might be recruited into the response, possibly bringing into play the lengthening reaction. Of course afferent responses which depend upon active tension would not appear at all in these experiments. Since stimulation of some, at any rate, of the smaller fibers of the gastrocnemius nerve yields reflex discharges directed into flexor nerves (14, Fig 7), it is a fair assumption that those fibers subserve nociception rather than proprioception. Certainly no responses resembling the group III reflex obtained by strong stimulation of the gastrocnemius nerves (14, Fig 7) have been realized on the occasion of brief stretch in the present experiments.

The latency of the stretch-evoked reflex at the ventral root in the experiments of this series has varied between 3.6 and 3.9 msec. In order to estimate the minimum total reflex latency for the tendon-jerk of the gastrocnemius muscle it is necessary to add to the latency at the ventral root sufficient time for conduction to the muscle and for neuromuscular delay. The additional motor conduction time is approximately 1.8 msec; neuromuscular delay is approximately 0.55 msec (11). The minimum total latency from the onset of stretch to the onset of the muscle action potential *at the end plate zone* would approximate 5.95 msec. This is somewhat shorter than the value 8.6 msec obtained by Denny-Brown (2). Since this value is based on the known transmission time through the minimum reflex arc, it is not expected that the minimum reflex time for the tendon-jerk can be further reduced, except inasmuch as some muscles are closer, anatomically speaking, to the spinal cord, and would involve less time in simple conduction. Other latency values for the most part have been obtained from the knee-jerk preparation rather than the ankle-jerk preparation (8, 5, 1, 2). The conduction pathway for the knee-jerk is shorter and that reflex should appear with shorter latency. The shortest latency encountered by Jolly was 5.3 msec (8).

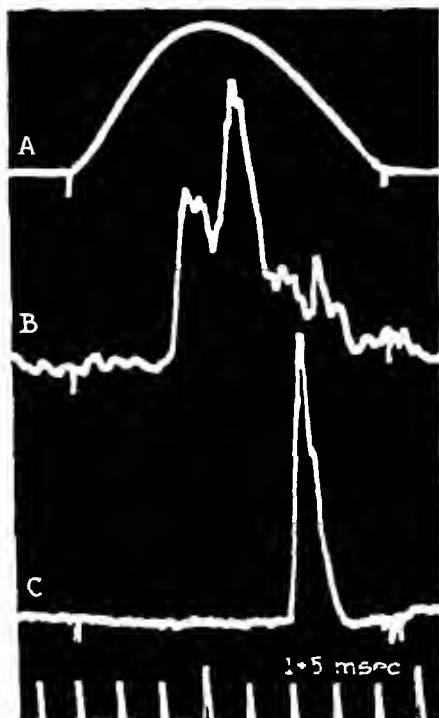


FIG 6 Tendon-jerk reflex as in Fig 5, but in another preparation. A—stretch imposed upon the gastrocnemius muscle. B—afferent response recorded from the S1 dorsal root. C—reflex response recorded from the S1 ventral root. The single reflex volley corresponds to the second reflex volley in the experiment of Fig 5. The initial afferent volley is subliminal for the motoneurons. Time in 1 and 5 msec intervals.



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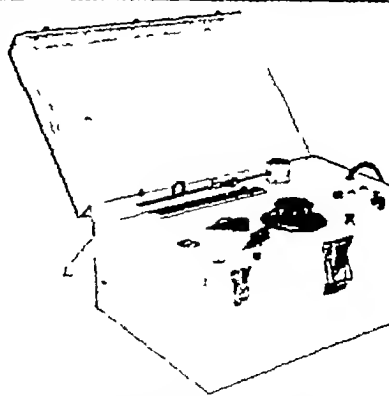
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## RESULTS

1 *ACh in normal cortex* MacIntosh (10) in a thorough analysis for ACh in the central and peripheral nervous systems of the cat and dog prepared his animals by injecting chloralose or chloralose-urethane and eserine. Extracts of tissues were made with trichloroacetic acid. In order to determine the most satisfactory methods to employ in the experimental studies a number of procedures were tried on normal rats to test the effects of anaesthesia, injection of eserine, and different methods of extraction. Nembutal anaesthesia, with and without eserine, was tried, and three extraction procedures were tested.

(a) *Dialysis* Rats were injected subcutaneously with 0.1 gram of nembutal. After deep anaesthesia the cortex was removed, weighed, and ground with silica in unbuffered frog heart Ringer (6.5 g NaCl, 0.14 g KCl, 0.12 g  $\text{CaCl}_2$ ,  $\text{H}_2\text{O}$  to 1 l.) plus eserine (physostigmine) sulphate 1/10,000. The volume was adjusted to 1 cc. for each 100 mg. of tissue. This was dialysed in a cellophane sac against an equal volume of unbuffered Ringer for 2 hours at 15°C. The fluid outside the sac was diluted with bicarbonate Ringer and assayed on the frog heart. Assay of the cortex from six rats gave an average value of 1.17  $\gamma$  ACh/gram of wet tissue. A similar procedure was repeated on four rats except that they received 1 cc. of 1/5000 eserine sulphate subcutaneously. This produced characteristic salivation and muscular tremors. Upon assay an average value of 1.35  $\gamma$  ACh/gram of cortex was obtained.

By using the Venus heart, which is apparently unaffected by substances other than ACh in tissue suspensions, at the great dilutions employed, it was possible to assay for ACh in the contents of the sac as well as in the surrounding fluid. A higher value of ACh was always found inside the sac even after several hours of dialysis. This suggested that some synthesis might be taking place during dialysis, or that some ACh was bound to large molecules which did not dialyze out, hence this method was considered unsatisfactory for the type of experiments to be done later.

(b) *Trichloroacetic acid extraction* Cortical tissue from five rats was extracted with 10 per cent trichloroacetic acid after the method of Chang and Gaddum (1). Assays were made on both the frog heart and the Venus heart. Those on the former were not always satisfactory, probably due to occasional incomplete removal of all traces of acid and ether which have a deleterious effect on the frog heart. The greater sensitivity of the Venus heart allowed greater dilution, and assays on this preparation gave an average value of 1.6  $\gamma$  ACh/gram of cortex. This may be considered a fair estimate of the total ACh in the rat cortex.

(c) *Cold-Ringer extraction* It was believed that the free\* ACh of the cortex might be more labile than the bound and might increase or decrease in amount when there was no measureable change in total ACh. Hence a

\* In the present discussion the "free ACh" may be defined as that part of the total ACh which is readily extractible in water in the presence of adequate amounts of eserine or prostigmine, but without employing a protein denaturant.

# ACETYLCHOLINE LEVEL OF RAT CEREBRAL CORTEX UNDER CONDITIONS OF ANOXIA AND HYPOGLYCEMIA

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## INTRODUCTION

THE IMPORTANCE of oxygen and glucose in the synthesis of acetylcholine (ACh) by brain slices has been demonstrated by Quastel, Tennenbaum and Wheatley (15) and by Mann, Tennenbaum and Quastel (11, 12). The *in vivo* synthesis of ACh by the superior cervical ganglion of the cat has also been shown to depend on an adequate supply of oxygen and glucose (7). However, attempts to demonstrate a change in the ACh level of the brain as a result of anoxia or hypoglycemia have thus far failed (9, 3). The present paper is an account of experiments which demonstrate, with reasonable certainty, that low atmospheric pressure (probably acting through anoxia), and insulin hypoglycemia, cause a decrease in the level of ACh in the cerebral cortex of the rat.

## METHODS

Rats of the Sprague-Dawley albino strain were used. These ranged in weight from 110 to 300 g but the majority weighed between 200 and 250 g. Anoxia was produced by subjecting rats to low atmospheric pressure. The chamber was evacuated in steps of 50 or 100 mm Hg with pauses of 10 minutes between successive lowerings. At a stage when respiratory movements had just ceased (between 100 and 200 mm Hg) the pressure was quickly returned to normal, the rat decapitated, and the cortex removed for extraction. Hypoglycemia was produced by fasting rats 16 to 22 hours, always including one night, then injecting insulin intramuscularly. Some were allowed to survive 30 minutes after the injection, some were sacrificed in convulsions and others in coma. All experiments were terminated by decapitation.

Three methods of extracting the ACh were tried. The trichloroacetic acid method of Chang and Gaddum (1) and a dialysis method after Lissák (8) were not found entirely satisfactory for reasons to be given later. A rapid, cold-Ringer method was developed which extracted the free ACh and this was used in the majority of the experiments. Three methods of assay were also tried: the leech muscle preparation, the isolated, eserized, frog heart (Straub preparation), and the isolated heart of the mollusc *Venus mercenaria*.† The leech muscle preparation was discarded as too slow and insensitive. In one series of experiments (Summer series) all extracts were tested simultaneously on the frog heart and the *Venus* heart by different persons. In the Fall series assays were made on only the frog heart, and by a third person. Each extract was matched several times with known concentrations of ACh. The stock solution of acetylcholine chloride was made up in 5 per cent  $\text{NaH}_2\text{PO}_4$ , sealed in ampoules, heated to boiling for 5 minutes and stored in the refrigerator. Stock ACh prepared in this manner undergoes about 10 per cent loss of activity in one year. All values for tissue ACh obtained in this investigation are expressed in terms of an equivalent weight of the free base and not of the chloride. Further details of procedure will be given as the separate experiments are considered.

\* With the technical assistance of Sibyl Beckett, Jane E. Hyde and Todd C. Smith.

† Aided by a grant from the Milton Fund of Harvard University.

‡ Details of the method of assaying tissue extracts for ACh, employing the *Venus* heart (based on findings of Prosser, 14), will be described in a separate publication.

Table 1 Acetylcholine levels of cerebral cortex of rats under conditions of low atmospheric pressure A Total ACh by trichloroacetic acid extraction B Free ACh by cold-Ringer extraction Summer Series C Free ACh by cold-Ringer extraction Fall Series

No of animals	Condition	Assays on Venus Heart			Assays on Frog Heart		
		Range	Average	S E	Range	Average	S E
A 5 5	Normal	ACh $\gamma$ /gm 0.9-2.8	ACh $\gamma$ /gm 1.6	0.32	ACh $\gamma$ /gm 0.25-1.0	ACh $\gamma$ /gm 0.46	0.15
	low pressure	0.4-2.0	1.1	0.29	0.10-0.80	0.28	0.13
B 9 8	Normal	0.12-0.60	0.36	0.057	0.13-0.40	0.26	0.04
	low pressure	0.10-0.28	0.17	0.023	0.10-0.25	0.15	0.02
C 6 6	Normal	—	—	—	0.20-0.75	0.35	0.10
	low pressure	—	—	—	0.13-0.45	0.23	0.09

A statistical analysis of the data of Table 1 shows that only in series B are the differences in the mean ACh values of normal and experimental rats significant, according to the usual method of determining significance. Both sets of assays give differences between the means which are more than twice the standard error of the difference.

In series A and C, which contain too few determinations for statistical analysis, in only two instances were the ACh values of the cortex of low pressure-treated rats the same as those of the normals tested on the same assay preparation. In one of these (rats 31 and 32, Table 2) the same extracts when tested on the Venus heart yielded a lower value for the cortex of the experimental rat. To illustrate this point the data of series A Table 1 are shown in Table 2.

There is little doubt that much of the apparent variation in ACh content of a given normal tissue which we, and others, have observed is due to the variability of the assay preparations. When cortical tissues of two or more normal rats are extracted and assayed on the same test preparation the values obtained are much more uniform than when the same extracts are

Table 2 Individual values for total ACh, (Series A—Table 1)

Assays on Venus Heart				Assays on Frog Heart			
Normal		Low Pressure		Normal		Low Pressure	
Rat	ACh $\gamma$ /gm	Rat	ACh $\gamma$ /gm	Rat	ACh $\gamma$ /gm	Rat	ACh $\gamma$ /gm
19	2.8	18	2.0	19	1.00	18	0.80
24	1.6	25	1.3	24	0.50	25	0.10
27	1.4	28	0.7	27	0.25	28	0.12
29	0.9	30	0.4	29	0.30	30	0.15
32	1.2	31	1.0	32	0.25	31	0.25
av 1.6		av 1.1		av 0.46		av 0.28	

quick method was evolved to extract the free fraction. The removed cortex was placed in 2 cc of ice cold, unbuffered frog heart Ringer with eserine 1:10,000, weighed, ground with silica in a chilled mortar and the volume adjusted to 1 cc for each 100 mg of cortex. The suspension was mixed well and centrifuged at high speed for 5 or 10 minutes. It was found that the length of time that the tissue suspension was mixed and allowed to stand before centrifuging affected the yield, hence for a given series this period was kept as constant as possible. After centrifuging, the supernatant fluid was poured off and stored at near 0°C until just before assaying, when it was diluted with bicarbonate Ringer, or sea water, depending on which heart preparation was to be used. With this procedure extracts were ready for assay within 20 to 30 minutes from the beginning of removal of the cortex.

Three rats under nembutal anaesthesia yielded an average value of 0.33  $\gamma$  free ACh per g of cortex when assayed on the frog heart and 0.43  $\gamma$  when assayed on the Venus heart. Lower values for a given extract were consistently obtained with the frog heart due to the presence of small amounts of excitatory substances. These had no apparent effect on the Venus heart.

Seven normal rats decapitated with no previous treatment, gave an average value of 0.25  $\gamma$  free ACh/gram of cortex when assayed on the frog heart and 0.35  $\gamma$  when assayed on the Venus heart. Comparing this value obtained on the Venus heart with that of the total ACh by the trichloroacetic acid method (1.6  $\gamma$ ) it is seen that the free ACh constitutes about one fourth the total ACh of the rat cortex. Mann, Tennenbaum and Quastel (11) found the free ACh in whole rat brain to be "less than 1.0  $\gamma$ /gram" and the total ACh to range from 1.0 to 2.9  $\gamma$ /gram.

In the account of experiments to follow, it should be noted that a control rat was nearly always run with each experimental rat and the two extracts assayed on the same heart preparations. Thus avoided, to some extent, variations resulting from small differences in extraction procedure and assay, which inevitably resulted from having several persons participating in the experiments, thus making differences between experimental animals and controls obvious when a series was not large enough to permit statistical analysis of the data.

2 *Low atmospheric pressure and ACh level of cortex* Three groups of rats without pretreatment were exposed individually for 1-2 hours to periodic decreases in atmospheric pressure until respiratory movements ceased. They were then decapitated and cortical tissue extracted and assayed for ACh. A total of nineteen rats were exposed to low pressure and twenty normal rats used as controls. The results are summarized in Table 1. It may be noted that the range of individual values for ACh in normal and low pressure rats overlap but in each group, and by both methods of extraction and assay, the average values for rats subjected to low pressure are lower than for a corresponding number of normal rats. The decrease in free or total ACh in the different groups and by the different assays varies from 31 to 52 per cent.

(average 2.3) Six rabbits given insulin gave values for hemispheres ranging from 1.1–1.7  $\gamma$ ACh/gram (average 1.3) while values for brain stem ranged from 1.9–2.4  $\gamma$ /gram (average 2.1). These authors likewise concluded that there was no effect of insulin hypoglycemia on the ACh level of the rabbit brain. Nevertheless it was considered worthwhile to repeat such experiments on the rat, measuring the free ACh rather than total ACh and using more sensitive test preparations than those employed by MacIntosh and by Cortell *et al*.

Rats were fasted for 16 to 22 hours, always including one night. Some of these were used as controls, others injected with insulin (5 or more units per

Table 3 Free acetylcholine levels of cerebral cortex of rats under conditions of insulin hypoglycemia A Summer Series B Autumn Series

No of Animals	Condition	Assays on Venus Heart		Assays on Frog Heart	
		Range	Average	Range	Average
A 5	Fasted only	ACh $\gamma$ /gm 0.30–0.45	ACh $\gamma$ /gm 0.34	ACh $\gamma$ /gm 0.20–0.25	ACh $\gamma$ /gm 0.23
	30 min after insulin	0.10–0.16	0.15	0.06–0.15	0.12
	convulsions	0.05–0.20	0.12	0.03–0.12	0.09
	coma	0.14–0.20	0.17	0.10–0.20	0.15
B 10	Fasted only	—	—	0.20–1.0	0.68
	convulsions	—	—	0.12–0.40	0.28
	coma	—	—	0.40–0.50	0.45

kilogram) Two series of experiments were performed independently by different persons. In series A some rats were killed 30 minutes after the injection of insulin, some in convulsions and some in coma. In series B seven were killed in convulsions, only two in coma. Extraction was by the cold-Ringer method with eserine.

The results are given in Table 3. That there is a marked decrease in the level of free ACh in the rat cortex as a result of insulin hypoglycemia is apparent. The greatest decrease is seen in the rats killed in convulsions. In the few rats killed in coma there is some apparent return toward normal levels but too few determinations were made for this to be a final conclusion.

The relatively higher values for ACh in both controls and hypoglycemic rats in series B compared with series A was due to a difference in extraction procedure. In B the triturated cortical tissue was allowed to stand longer before centrifuging than in A.

5 *In vitro experiments* Five rats were decapitated in insulin convulsions and the cortex divided in approximately equal halves. These were weighed and minced finely in sufficient phosphate-Locke solution (11) containing eserine sulphate 1:10,000, to give 100 mg of cortex per cc. Each lot was placed in a tube from which the air could be evacuated and glucose was

assayed on different test preparations. It was, for this reason, that an extract of the cortex of a normal rat was assayed with that of each experimental rat, on one or more test preparations, and when consistent differences were found they were considered significant.

3 *Prostigmine treatment and low pressure* If the effect of the low pressure in decreasing the level of ACh in the rat cortex were due to the breakdown of ACh by cholinesterase, in the normal functioning of the brain, and failure to synthesize new ACh due to oxygen lack, it should be possible to prevent or reduce the loss by previous administration of an anti-cholinesterase. Since prostigmine is one of the most effective of such agents it was employed to test this idea.

Pairs of rats were injected subcutaneously with 0.1 or 0.2 cc of a 1:5000 solution of prostigmine (Prostigmin) bromide (Hoffmann-LaRoche)\*. The general reactions of the rats to this amount of prostigmine were comparable to those produced by approximately five times as much eserine. One rat was subjected to the same low pressure treatment as in section 2 above, the other used as a control. Ten pairs of rats were so treated. Extracts were made by the cold-Ringer method except that prostigmine was used as an anti-cholinesterase instead of eserine. Assays were made on frog hearts only. The ten control rats kept for 1-2 hours after the injection of prostigmine gave values for free ACh ranging from 0.2 to 0.7  $\gamma$ /gram of cortex with an average value of 0.44  $\gamma$ . The rats receiving prostigmine and the low pressure treatment yielded values ranging from 0.2 to 0.75  $\gamma$  ACh with an average value of 0.43  $\gamma$ /gram. Such close agreement between the controls and the low pressure rats may be quite accidental, nevertheless one would conclude that treatment with prostigmine does prevent the decrease in ACh found in rats subjected to low pressures without previous administration of this anti-cholinesterase.

Observations of the prostigminized rats while being subjected to low pressure failed to reveal any obvious increased tolerance to the low pressure when compared with rats without prostigmine. To check this, a number of rats were subjected to low pressures repeatedly—one day without prostigmine and the following day with. No constant differences in the behavior of the same individual rat could be observed.

4 *Insulin hypoglycemia and ACh level of cortex* MacIntosh (9) determined the total ACh in whole brains of seven normal mice and obtained values ranging from 1.5-2.6  $\gamma$  ACh/gram. Six mice killed in insulin convulsions gave values ranging from 1.5-1.8  $\gamma$ /gram. However, he concluded that insulin hypoglycemia has no effect on the ACh level of the mouse brain. Cortell, Feldman and Gellhorn (3) performed similar experiments on rabbits, making assays on the eserminized rectus abdominis muscle of the frog. From eight normal rabbits they obtained values for hemispheres ranging from 1.0-2.0  $\gamma$ /gram (average 1.5), and for brain stem, values ranging from 2.0-2.7  $\gamma$ /gram.

\* Supplied through the kindness of Hoffmann-LaRoche, Inc.



suggests that the decrease in ACh resulting from anoxia or hypoglycemia is responsible for the decreased level of excitability of the cortex

### SUMMARY

1 Several methods of extraction and assay of free and total ACh in the cerebral cortex of the normal rat are compared

2 Subjecting rats to low atmospheric pressure for 1 to 2 hours is shown to decrease the level of free or total ACh in the cortex by approximately one third to one half

3 Administration of prostigmine before low pressure treatment prevents a decrease in free ACh in the cortex

4 Insulin hypoglycemia results in a greater decrease in free ACh than that produced by the low pressure treatment

5 It is suggested that the decline of free ACh may account for the decrease in excitability of the cortex under conditions of anoxia and hypoglycemia

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added to give 0.02 M glucose. One tube was evacuated and sealed, the other was left exposed to air. Both lots were incubated at 37°C for 2.5 hours with occasional shaking. At the end of this time the contents were centrifuged and the clear centrifugate assayed on the frog heart. Values for free ACh of the cortex incubated with air excluded ranged from 0.1 to 0.2  $\gamma$ /gram. Corresponding halves of the cortex incubated in air gave values approximately ten times as great.

This agrees with the observations (11, 15) that synthesis of ACh *in vitro* is an aerobic process and demonstrates that certain precautions must be observed in the measurement of ACh in tissues of anoxic animals. If, in the extraction process, tissues are allowed to remain at room temperature or higher, with air available, a considerable amount of synthesis of ACh may occur if substrates are present, thus restoring the level to a more or less normal amount. It is believed that by carrying out the extraction in the shortest possible time and at a temperature near 0°C this is largely obviated.

### DISCUSSION

From the above results it appears that low atmospheric pressure (probably acting through anoxia) and insulin hypoglycemia produce a decrease in the level of ACh in the cerebral cortex of the rat. While earlier investigators (3, 9) concluded that there was no reduction in total ACh in the hypoglycemic or anoxic mouse or rabbit brain, an examination of the data shows that their highest values for treated animals were, in no case, as large as normal values. Therefore it may be concluded with reasonable certainty that glucose and oxygen are important in the *in vivo* synthesis of ACh as well as for *in vitro* synthesis as had been demonstrated by earlier investigators.

The precise role of ACh in the brain is not known, however this finding that the level of ACh is modified by low oxygen and low blood sugar helps in the understanding of certain observations on electrical activity in the cortex. It has been demonstrated (5, 6, 16 and others) that almost any procedure which alters the supply of glucose to the central nervous system, or the oxidation of the glucose, alters the electrical picture in the brain. In hypoglycemia there is a decrease in alpha waves and an appearance of large slow delta waves indicating a loss of excitability. At the time of convulsions the cortex is "silent" or nearly so (4).

The opposite occurs when eserine or prostigmine (or ACh after one of these) is applied to the cortex. Miller, Stravinsky and Woonton (13) applied eserine to the rabbit cortex and noted the reduction in amplitude of slow waves and the appearance of small fast waves. ACh after eserine in the rabbit and cat caused the disappearance of slow waves and the appearance of spikes of high amplitude and frequency. Chatfield and Dempsey (2) applied ACh after prostigmine, to somesthetic, auditory and motor areas of the cat cortex and noted increased spontaneous activity and the appearance of 5-10/sec spikes followed by 20-30/sec low voltage potentials. This

poptiteal nerve where they enter the fleshy portion of the muscles below the knee. Sometimes the whole sciatic stem was used.

The micro-electrode was of the type used in this laboratory (14) for work on the retina and consisted of a fine platinum wire insulated by glass. It is pressed vertically against the dorsal roots not far from the point where they enter the cord. Some fibres are then found to be spontaneously active, others silent, some again are incited to activity by the pressure of the micro-electrode. Spontaneous discharges may or may not be accelerated by the stimulus. When relatively thin muscular branches are stimulated it is difficult to find an active fibre. It is also easier to locate a diffuse discharge in response to stimulation than to succeed in isolating a single spike. It is possible, in cases when a large spike suddenly turns up in perfect isolation, that it represents a certain amount of synchronized activity. Still, the discharges look and behave like so-called single units. Their often less strict correspondence with the all-or-none law can be explained by the influence of the discharge in adjacent elements.

A time signal interrupts the beam for marking the stimulus. Dependent upon what is being pictured this beam is interrupted at a rate of 50 or 500 per sec.

## RESULTS

*All-or-none nature of response.* Only if several active fibres are lying under the electrode can the dorsal root reflex (16, 22), conducted centrifugally from the spinal cord, be a source of error. However, the latent period of this discharge is much longer than that of the direct centripetal volley. But, when the micro-electrode is used, the chance of finding a centrifugally conducting fibre is practically *nil*. It is difficult to isolate a directly excited single spike.

In Fig. 1 are illustrated the effects of a variation in (1) strength of stimulus at constant rate of rise and (2) length of plateau at constant strength.

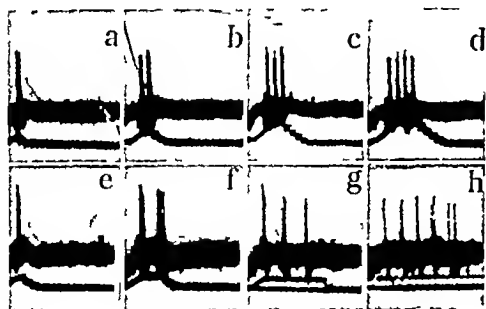


FIG 1 Superimposed pictures of several sweeps with the cathode ray. Form and strength of stimulus on lower cathode ray. Time in 500/sec. From a to d stimulus increases in strength at constant rate of rise, a, threshold, h, 2.4 rheobases, c, 3.7 rheobases, d, 5.0 rheobases. From e to f lengthening of duration of plateau at constant strength of the stimulus and constant rate of rise.

and rate of rise. In both cases the same stimulus pattern was swept several times across the screen of the tube. In none of the later pictures has this particular technical arrangement been used. Figure 1 is reproduced to show the all-or-none manner in which impulses are added up to a rhythmic discharge. With less perfect isolation the spikes diminish when strength or gradient is diminished. Both variations, in strength from a to d, and in plateau length from e to h, show that spikes are added with such regularity that the whole process can be swept across the tube several times without any other effect than a slight increase in width of the spikes, indicating minor variations of latent period. Our technique is thus sufficiently discriminative for the purpose for which we intend to use it.

# ACCOMMODATION AND AUTORHYTHMIC MECHANISM IN SINGLE SENSORY FIBRES

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THE TECHNIQUE for measuring accommodation directly in relation to the discharge of impulses, developed in this laboratory by Skoglund (21), makes it possible to correlate a number of properties of nervous activity with the accommodation curves. The latter serve as reference point in the analysis which also by this method is shifting emphasis from measurements of threshold variations to inspection of the discharge as such and measurements of its properties. A further improvement upon Skoglund's work is introduced in this paper by the use of micro-electrodes, placed directly on the sensory roots in order to isolate single fibres in nerves electrically stimulated with linearly rising currents. This technique is here used for the purpose of studying the properties of the autorhythmic mechanism in sensory fibres of different accommodative resistance.

Bernhard, Granit and Skoglund (6) proposed as a working hypothesis that the electrotonic potential, seen in spinal roots by Barron and Matthews (5) and studied in the optic nerve by Bernhard (7, 8, 9) serves as exciting for the autorhythmic mechanism in the nerve itself. From this and allied points of view we need more information about the properties of the rhythmic discharge caused by stimulation with slowly rising currents imitating possible generator potentials conducted electrotonically from the axon hillock down the fibres.

## TECHNIQUE AND PROCEDURE

*Stimulation* The stimulating device, built by the physicist of this laboratory, Mr K T Helme, has been described in detail by Skoglund (21). The apparatus delivers linearly increasing currents of strictly controllable gradient and strength through the anode circuit of a valve. This stimulator is connected so as to shift the one beam of a double cathode ray in proportion to the rate of rise of the stimulating currents, the other beam being used for simultaneous records of the discharge in the nerve through a condenser coupled amplifier (see the figures of this paper). Strength and gradient are independent variables. The stimulus was driven up to a certain strength at a certain rate, then left at plateau height for some time, and finally allowed to drop back at a rate of fall corresponding to its rate of rise. If the plateaus were brief, the stimulator was operated iteratively by a sweep circuit. With longer plateaus it was necessary to start the stimulator manually. Plateaus from a few milliseconds to some 60 seconds were used.

*Preparation* The spinal cord of decerebrate cats was laid bare and the animals slightly lifted up in the preparation box by a specially designed clamp gripping firmly around one thoracic and one sacral spinous process and rigidly fixed to a heavy stand. These precautions are necessary for the sake of the micro-electrode. Without a clamp on the vertebrae themselves each respiratory movement is accompanied by considerable excursions of the preparation under the electrode. Fine adjustment of the latter was achieved with the aid of a micromanipulator. Well chlorinated silver-silverchloride electrodes were used for stimulation. In order to differentiate between nerves from the cutaneous and the muscular end organs these electrodes were either placed on the saphenous nerve or on twigs from the

*Two phases of rhythmic discharge "Silent period"* It has been pointed out previously (11, 21) that the rhythmic discharge can be divided into two phases, initial spikes of high frequency and a later prolonged discharge. We have now an opportunity of finding out whether these phases occur in a single element or represent different fibres. From this point of view Fig 3 is of particular interest. The rate of rise is constant and the final strength of the current varies from a to f. Record g is from another experiment.

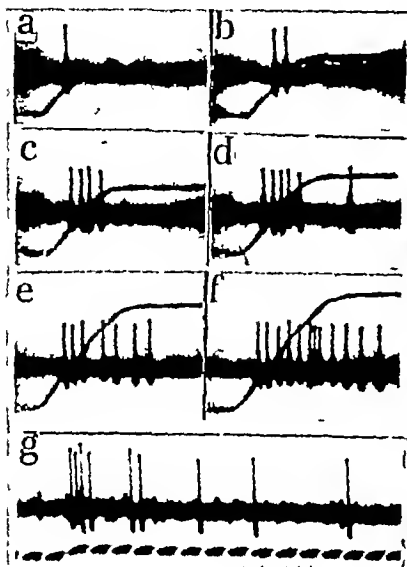


FIG 3 Muscular afferent Stimuli rising from a to f at constant rate of rise to greater strength. a, 1.4 rheobases, b, 1.6, c, 2.0, d, 2.4, e, 3.2 and f, 4.0 rheobases. Record g from another experiment illustrates "silent period".

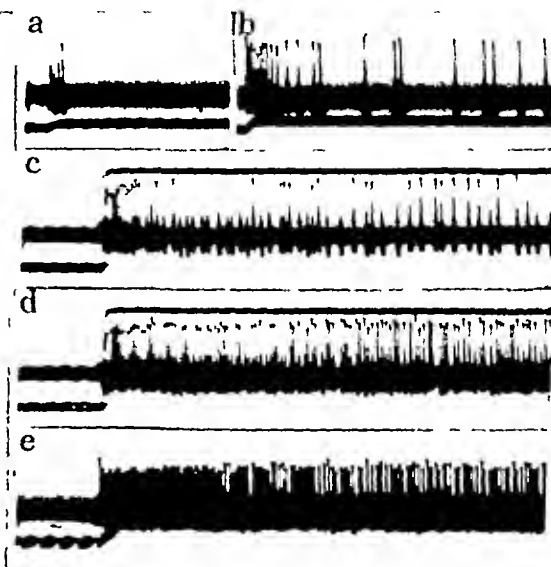


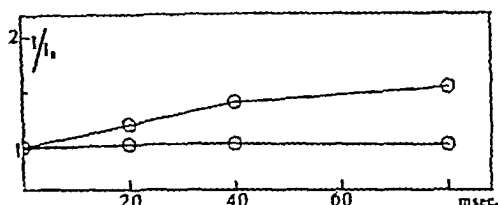
FIG 4 Muscular afferent Strength of stimulus increases from a to e (see text). The plateau frequencies are a, 0, b, 75, c, 140, d, 275 and e, 450 per sec. These are counted after about 20 msec of stimulation. Time in 50/sec.

The development of the initial phase of the discharge is seen in the records a to d. Continuation of the rising phase of the current is accompanied by the birth of new impulses. In record d, however, there follows after a silent period the first impulse of the later plateau-phase of the discharge. Still stronger stimuli, as in e and f, do not lead to a silent period because the threshold for the plateau-phase is then reached during or just after the rising portion of the stimulus so that it becomes submerged into the initial phase. With linearly rising stimuli the occurrence of a silent period between the two phases of the discharge is left to chance but it could, no doubt, be produced regularly if with our apparatus it were possible to decrease the rate of rise of the stimulus somewhat at the top. In record g the silent period is very prominent.

**Accommodations curve** In order to plot accommodation curves (21) the actual stimuli of different rates of rise are drawn in a co-ordinate system in which the abscissae accordingly are rising times and the ordinates strength. These values are obtained directly from the photographed curves (see figures) in which the final plateau level of current always was checked by a milliammeter in the circuit. Thus one does not merely rely on the amplified deflexion of the "stimulus-beam" of the cathode ray. With a large range of stimulus intensities tested, it is often necessary to alter the degree of amplification in order to keep the beam within the proportionality range of the instruments.

The various stimuli having been inserted into the co-ordinate system, the moment of appearance of the *first* spike is marked on the curve for each linearly rising stimulus. The points so obtained are joined to a curve (see Fig. 2) which accordingly illustrates the strength to which it has been neces-

FIG. 2 Average accommodation curve for muscular afferents (upper curve) and for the saphenous (lower curve). Ordinates multiples of rheobasic strength ( $I/I_0$ ) (see text)



sary to drive the slowly rising current at each particular gradient in order to elicit *one* impulse, the first of a series, or a single spike if others do not follow. For rapidly rising stimuli the conduction time must be subtracted.

The graph so obtained is of the same type as the one introduced by von Kries (18) and also used by Hill (15) and his collaborators. Depending upon the particular problem in view, strength of current ( $I$ ) may be given in milliamp or in multiples of rheobasic strength ( $I/I_0$ ). The inverse value of the rate of rise of the initial rectilinear portion of the curve corresponds to Hill's constant  $\lambda$ . However, Skoglund's direct method of measuring accommodation and plotting the curve from photographed responses eliminates a number of errors and difficulties inherent in the methods based on observation of a threshold muscle contraction as "constant" index (6, 21) and makes it possible to isolate single fibres, not to mention the advantage of also being able to study sensory nerves by the direct method.

In Fig. 2 are plotted the average accommodation curves for muscular afferents and the purely cutaneous saphenous nerve (A fibres). The latter curve hardly rises at all above the rheobase. The muscular afferent also has relatively little accommodation, compared with motor nerves (12, 21). Breakdown of accommodation follows at about 1.5 rheobases. Hill's constant  $\lambda$  was about 150–200 msec with muscular afferents and approaching infinity with the saphenous. These differences, as we shall see, are large enough to reappear mirrored in the properties of the autorhythmic mechanism of the nerves.

gion 400–500 We conclude that variations in accommodative resistance within the limits of these experiments have no definite influence on the strength-frequency relation

*Adaptation and accommodation* By adaptation is meant the fact that the frequency of the discharge gradually sinks during prolonged stimulation at plateau height Figure 6 illustrates different stages of this process during the course of stimulation with a current 5.8 times the rheobase This nerve

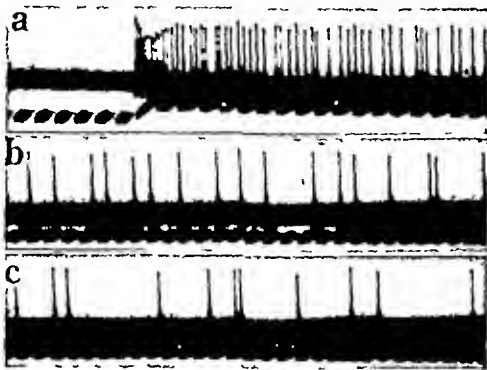
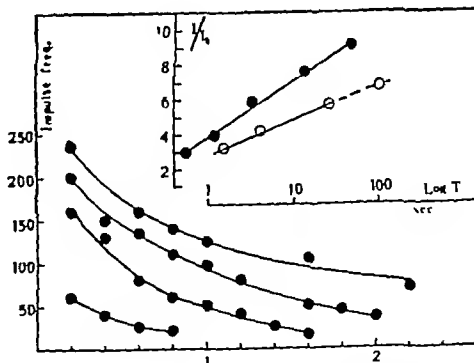


FIG 6 Muscular afferent The course of adaptation to plateau strength of a stimulus 5.8 times the rheobase Record begins in a with about 250 impulses per sec In b, after 2.5 sec, it has fallen to 35/sec, in c, after 5.0 sec, to 18/sec

is a muscular afferent With the saphenous we have often kept the stimulus at plateau height for 60 sec and still seen a discharge of considerable frequency But in nerves with some accommodation the frequency much sooner

FIG 7 Muscular afferent Course of adaptation for stimuli of different strength in terms of frequency of discharge as ordinates against duration of stimulation as abscissae Inset Comparison of a muscular afferent (filled circles) of  $\lambda = 200$  with a saphenous (open circles) of  $\lambda = \infty$  with respect to the total adaptation time  $T$  for the rhythmic discharge at strengths of stimulation given as ordinates in multiples of the rheobase  $\log T$  on the abscissa Stimulation has been continued until the rhythmic discharge has stopped



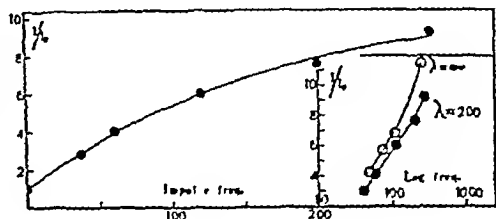
reaches zero In such cases the total adaptation time ( $T$ ) can be measured

A set of curves illustrating the decline in frequency from different levels of excitation ( $I/I_0$ ), defined by the initial plateau frequencies, is found in Fig 7 The abscissae show the duration of the plateau stimulus The nerve is a muscular afferent In the inset of Fig 7 multiples of rheobasic strength ( $I/I_0$ ) are plotted against the logarithm of the total adaptation time ( $\log T$ ), i.e. the time from the first to the last impulse of the discharge caused by

Previously we (6) have defined as "breakdown of accommodation" the fact that at a certain strength of current its strength alone rather than its rate of rise becomes significant (see Fig 2) and pointed out that this intensity region is recognized by the flattening of the accommodation curve accompanied by iterative firing. The plateau discharge belongs to this late portion of the accommodation curve. Consequently this is the most important phase of the autorhythmic mechanism in sensory nerves which have low accommodative resistance. In the saphenous nerve it sets in practically as soon as the current has risen a little above rheobasic strength, in muscular afferents there is already a definite initial phase. But on the whole our preparation is more suitable for studying the plateau-phase. Motor nerve should be used for the initial phase. Gradient is there relatively more important.

In sensory nerves the initial phase seems to share with the plateau-phase the property of being more dependent upon current strength than upon its

FIG 5 Muscular afferent. Current strength in multiples of the rheobase as ordinates against plateau frequency of the discharge as abscissae. Inset: Same ordinates against log frequency in order to compare a saphenous nerve with a muscular afferent.



gradient. The range of variation in the frequency of the discharge is far greater during the plateau-phase than in the initial phase.

**Plateau discharge and current strength.** It is known that in general the frequency of the repetitive discharge is greater for stronger stimuli (12, 13, 17, 21). In Fig 4 is illustrated the effect of current strength on a single muscular afferent with  $\lambda = 200$ . On account of the large intensity range it has been necessary to use different degrees of amplification of the stimulus-beam of the cathode ray so that the deflexions are not proportional to current strength. In a, at 2.1 rheobases, there is merely the initial phase; in b, at 3.9 rheobases, the plateau-phase is well developed. In c the strength is 5.8 rheobases. The initial phase is from the very first record activated at a frequency high enough to cause diminution of the size of spikes. In d, at 9.7 rheobases, the plateau-phase is activated at a frequency leading to a relatively extended depression of spike size, and in e, at 15.0 rheobases, the spikes are subnormal for the whole period illustrated. The maximal frequency is at about 450 per sec.

In Fig 5 the quantitative relation between spike frequency and current strength in multiples of rheobasic strength ( $I/I_0$ ) is illustrated. In the inset the abscissae are log frequency and the strength-frequency relation has been compared for a muscular afferent and a saphenous. The difference between these two curves does not exceed the range of variation in different experiments. The asymptote of the curves is found to begin in the frequency re-



gion 400-500 We conclude that variations in accommodative resistance within the limits of these experiments have no definite influence on the strength-frequency relation

*Adaptation and accommodation* By adaptation is meant the fact that the frequency of the discharge gradually sinks during prolonged stimulation at plateau height Figure 6 illustrates different stages of this process during the course of stimulation with a current 5.8 times the rheobase This nerve

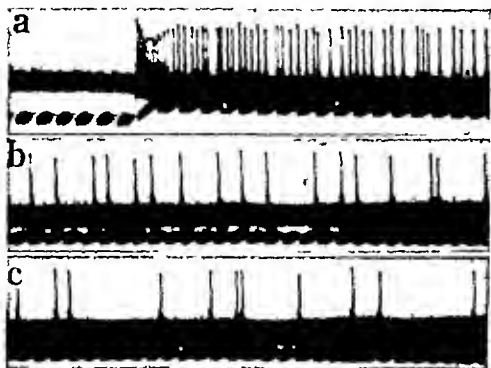
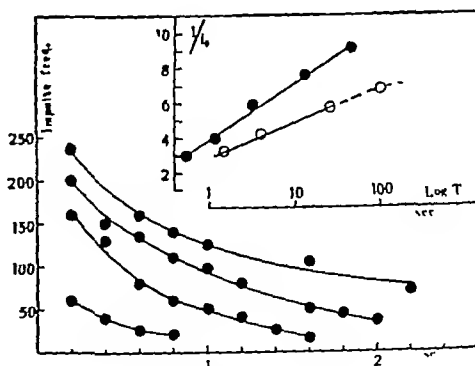


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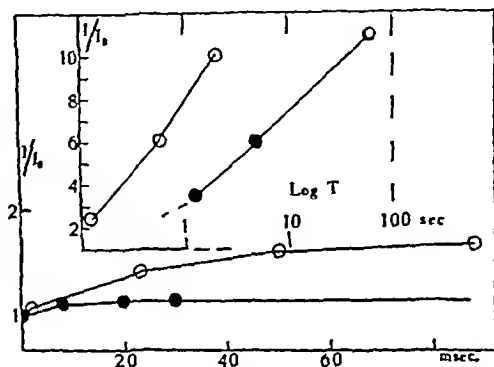
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a certain stimulus Here, as in all our experiments, the total adaptation time is a function of the accommodative resistance The steeper the rise of the accommodation curve, the steeper also the rise of the curve in the  $I/I_0$ -log T graph, the shorter consequently the adaptation time for a given multiple of rheobasic strength

This difference is well brought out in the inset of Fig 7 by the comparison of the muscular afferent of  $\lambda=200$  with the saphenous of a  $\lambda$  approaching infinity It is further emphasized by an interesting experiment in which the accommodation gradually rose while we succeeded in keeping the same fibre for several hours under the micro-electrode Hill's constant  $\lambda$  was about 200

FIG 8 Accommodation curves for a muscular afferent in the beginning (filled circles) and at the end (open circles) of an experiment (see text) Inset Plot of log T (abscissae) against multiples of rheobasic strength (ordinates) as in inset of Fig 7 Curve to the left thus belongs to nerve giving late accommodation curve ( $\lambda=85$ ), curve to the right to nerve giving early accommodation curve ( $\lambda=200$ )



in the beginning of the experiment and around 85 some hours later The two accommodation curves from which these approximated constants were obtained (from the early portion of the curve, as always) are shown in Fig 8 In the inset of the same figure are plotted the logarithms of the total adaptation times (log T), just as in the inset of Fig 7 The plot shows that the shortening of the adaptation time and the consequent steeper rise and shift of the  $I/I_0$ -log T curve actually is a function of the accommodative resistance of the active fibre and not due to differences in structure between muscular afferents and the saphenous, of a character unconnected with accommodation Both curves here refer to the same single muscular afferent fibre in different states of accommodation

*Adaptation and cathodal depression* Turning now to the original record of the experiment, evaluated above in Fig 8, we find in Fig 9 the late stage of it, characterized by  $\lambda=85$ , which should be compared with the records in Fig 6 from the early stage ( $\lambda=200$ ) In Fig 6, at 5.8 rheobases, there was still a plateau discharge present 5.0 sec after initiation of the rhythmic activity In record b of Fig 9, at 6.0 rheobases, the discharge stops after a little over half a second It does not last very much longer when the strength is increased in c to 9.0 rheobases The reason for this becomes evident when the current is further increased, to 14 rheobases in d The whole plateau discharge is now inhibited and possibly also the last portion of the initial phase This is the cathodal depression, noted by Schiff (20), then seen

The total adaptation time is thus of an order of magnitude which suggests, by decreasing with  $I/I_0$ , that quantitative agreement with the theory in sensory nerves only can be expected below the double rheobase. Nevertheless the theory has been a valuable instrument in the experimental analysis.

However, accommodation appears in an important function as common denominator for the general resistance of the nerve to impulse production. The greater this resistance, the more developed the cathodal depression, and the shorter the adaptation time which probably, as stated, is determined by the development of the Schiff-Wengé inhibition. This is an instance of what is meant by our statement in the introduction that accommodation serves as reference point in the analysis.

Whether this late cathodal depression can be equated with the early cathodal depression, studied by Erlanger and Blair (10, 12), remains to be found out in work with motor nerve where accommodative resistance is very much better developed. But it is clearly important that in their work too, accommodation, the early cathodal depression and the degree of repetitiousness are connected in the manner in which they now appear connected here (cf. also 21) despite the different mode of attack. Early cathodal depression is synonymous with accommodation as measured by Erlanger and Blair by a method based on testing the cathodal excitability with a shock technique.

*Autorhythmic discharge.* The fact that the sensory nerves, which are activated individually by isolated peripheral structures with limited energy resources, possess little accommodative resistance by comparison with motor nerves (12, 21) is in agreement with our hypothesis that the autorhythmic mechanism of these nerves can be put into operation by generator potentials carried down electrotonically along the nerve in simple end organs probably by direct chemical excitation of the nerve fibre. From this point of view it is interesting that particularly low accommodation and little adaptation is found in a cutaneous nerve such as the saphenous in which, on the theory, the autorhythmic mechanism would be started by energy released by such weak stimuli as touch or the bending of a hair. However, the degree of adaptation is clearly determined by the end organ and not by the mechanism in the nerve, as directly demonstrated by Adrian, Cattell and Hoagland (3) for cutaneous organs. In the nerve, adaptation of the autorhythmic mechanism is less marked in the saphenous, and clearly noticeable in the muscular afferents whereas the adaptabilities of the corresponding end organs are the other way round (1).

The fact that "gradient" is of relatively little significance in sensory nerves but highly important for many sense organs would seem to throw some light on the differentiation of such sense organs into a primary apparatus for picking up the energy of the adequate stimulus and a secondary generator mechanism firing the nerve. Sufficiently strong stimuli may, however, cause a "gradient" also in the generator mechanism and lead to a discharge interrupted by a "silent period" (initial phase followed by plateau-phase).

The activation of such a dormant tendency to intermittent conduction is shown in Fig 10. The stimulus seems to facilitate a process on the verge of activity. Similar observations have been reported by Erlanger and Blair (11). Barron and Matthews have emphasized the significance of their own observations for central inhibition. Like most central phenomena, this also seems to have its peripheral counterpart. Barron and Matthews' explanation could be adapted to fit our case but there is little reason to theorize here about observations which have been wholly unsystematical.

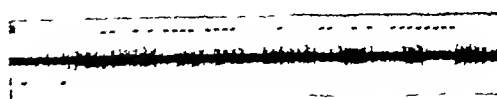


FIG 10 "Intermittent conduction"  
Stimulation of muscular afferent elicits discharge which soon is split up into regular groups

### DISCUSSION

*Adaptation time, accommodation and cathodal depression.* Hill's theory (15) explains the occurrence of rhythmic discharges to constant currents by the assumption that the "local potential"  $V$  for a certain time  $T$  remains above the "threshold"  $U$ . In the words of Katz (17) "during a certain time  $T$ , therefore,  $V$  will be greater than  $U$ , and throughout this time repetitive response might be expected to occur, at intervals determined by the refractory period." Stronger stimuli increase the frequency by eliciting impulses earlier in the refractory period.

The relation between this time  $T$  (which here has been called total adaptation time), the constant  $\lambda$ , and stimulus strength  $I/I_0$  has also been given by Katz. It is  $T = \lambda \log_e I/I_0$ . In frogs (whole nerve) he finds good agreement between experiment and theory. The reason for this may be the limited range of intensities tested. It is immediately seen that in our experiments with a value for  $\lambda$  around 200 msec  $T$  may be of the order of 1000 msec, for  $I/I_0$  around 3-4. The formula does not therefore fit our case. It may nevertheless be of some interest to compare the curves for the sensory fibre (Figs 8 and 9) in which  $\lambda$  was 200 in the beginning and 85 at the end of the experiment. Calling the adaptation times  $T_{200}$  and  $T_{85}$  for  $\lambda_1 = 200$  and  $\lambda_2 = 85$ , it is clear that on the theory

$$T_{200} - T_{85} = \log_e I/I_0 (\lambda_1 - \lambda_2),$$

the difference between the two constants being 115

The right and left members of this equation are compared in Table 1 for different values of  $I/I_0$ .

Table 1

$I/I_0$	$115 \log_e I/I_0$	$T_{200} - T_{85}$
10	262	38 000
6	206	4 400
4	160	1 500
2 extrapolated	79	0 230

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Considering the difference in accommodation between motor and sensory nerves it is clear that in reflex activity the transition from the one system to the other suggests interesting possibilities for selection on the basis of differences in accommodative resistance in the internuncial and output channels of the central nervous system

### SUMMARY

The repetitive discharge in response to slowly rising linear stimuli has been recorded with the aid of micro-electrodes from cutaneous and muscular afferents. Single fibres could be isolated by placing the micro-electrode on the dorsal roots. Stimulus form and nerve response are pictured simultaneously with the aid of a double cathode ray oscillograph on the same film.

By this method it is possible to measure the sensory accommodation curves directly and at the same time correlate them with the properties of the iterative discharge.

There is little if any accommodation in *n. saphenous*, representing cutaneous afferents (Hill's constant  $\lambda$  approaching infinity). For different muscular twigs of *n. popliteus* the values for  $\lambda$  range from 150 to 200 msec.

The autorhythmic discharge caused by the slowly rising stimuli consists of an initial phase during the time the stimulus rises and a later plateau-phase when the stimulus has reached a certain plateau level of strength. These two phases may be separated by a "silent period."

The plateau discharge is characterized by a frequency which increases with stimulus strength. The strength-frequency curve for single fibres is illustrated in Fig. 5. It is independent of the accommodative resistance of the nerve.

The total adaptation time (from first to last impulse) of the plateau discharge is a function of accommodation and of stimulus strength and decreases when the accommodative resistance increases or stimulus strength decreases. These relations are illustrated quantitatively for nerves of different accommodation in Fig. 7 and 8.

Strong stimuli continued on plateau height inhibit the discharge (Schiff-Wergo's cathodal depression), provided that the nerves possess good accommodative resistance. It is suggested that the total adaptation time is largely determined by this factor.

The slowly rising stimulus sometimes causes a rhythmically grouped discharge instead of a continuous flow of impulses.

We are indebted to the Rockefeller Foundation for a grant to this laboratory.

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area 8, of area 9, or of areas 10-11-12 produced some increase in activity but always short of that produced by removal of the whole frontal association area. The figures in both these papers indicate that the hyperactivity was usually delayed in onset and Kennard *et al.* emphasize the variability in the time of onset. Others have observed hyperactivity in the monkey (16, 23).

For the cat the situation is roughly similar. Barris (3) failed to observe hyperactivity following bilateral removal of the rostral portions of the neocortex but rather described symptoms of a cataleptic nature. Magoun and Ranson (15) were unable to confirm this latter finding but instead obtained hyperactivity. Langworthy and Richter (13) noted increased spontaneous activity in 9 of 10 animals in which they ablated the electrically excitable motor cortex, the premotor cortex and possibly a small tip of the corpus striatum. In earlier experiments, Langworthy and Kolb (12) destroyed various regions of the frontal cortex in 25 cats. Ten of these became hyperactive and the critical area was ventrolateral to the motor area. In the rat, Richter and Hawkes (20) found that unilateral as well as bilateral removal of the frontal pole (cortex and tip of the striatum) consistently increased activity. On the other hand in Beach's (4) series of 9 rats with lesions of the anterior cortex, only 6 became hyperactive and 3 became hypoactive. Striatal lesions were without consistent effect (5).

While only the most recent workers have recorded hyperactivity objectively, the phenomenon is so specific and outspoken, at least in monkeys, that it could scarcely be missed. The inconstancy of hyperactivity is therefore not likely to be due to any difficulty of observation. It is more probable that some variation in the lesion is responsible and this could be either an omission of a critical area or the inclusion of some region productive of apathy or hypoactivity. A consideration of the types of frontal lesions studied with respect to hyperactivity led us to the belief that the variable factor from lesion to lesion lay in the amount of destruction of the orbital surface of the frontal lobe. This present series of focal ablations on this surface indicates that area 13, which has recently been delineated cytoarchitecturally by Walker (22) is of especial importance in the production of hyperactivity.

#### METHODS

*Macaca mulatta* monkeys (T T Series, Nos 13, 14, 16, 21, 24) were used exclusively. The operations were performed aseptically under intraperitoneal sodium amytal anesthesia. A horse-shoe shaped skin-flap was reflected frontally and a transfrontal osteoplastic bone-flap was hinged on the temporal muscle. The dura was incised along the margins of the bone-flap, the superior longitudinal sinus was ligated and sectioned at its anterior extremity and the falx and the olfactory nerves were divided. Shifting the animal to the supine position allowed the brain to fall well away from the orbital plate. The pia-arachnoid was coagulated with the Bovie unit along the margins of area 13, which were laterally the fronto-marginal sulcus, medially the orbital sulcus, posteriorly the lateral olfactory striae, anteriorly the orbital gyrus was transected at the level of the bifurcation of the orbital sulcus. The bloc extirpated is roughly one sixth of the orbital face of the frontal lobes.

Activity was recorded pre- and postoperatively in the apparatus devised by Kennard, Spencer and Fountain (11). This is an oblong cage 4 ft by 1 25 ft and 1 25 ft high, set

# THE RELATION OF AREA 13 ON ORBITAL SURFACE OF FRONTAL LOBES TO HYPERACTIVITY AND HYPERPHAGIA IN MONKEYS\*

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HYPERACTIVITY is one of the principal objective consequences of bilateral prefrontal lesions, it has been described for representative animals from the rat to the monkey. But it is often not realized that hyperactivity is an inconstant result of prefrontal lobe lesions (4), and no adequate explanation of the inconstancy of its appearance is at hand. The time of onset of heightened activity is also exceedingly variable, being often delayed as much as three weeks and sometimes preceded by a period of depressed motility not ascribable to paresis. Neither is it known whether any component area of the prefrontal lobe is especially significant in the production of hyperactivity, some believe that prefrontal lobectomy alone gives maximal hyperactivity while others have designated special areas. On none of these points is the literature entirely unequivocal.

Bianchi (6) and Franz (7) made passing mention of hyperactivity in their protocols of monkeys subjected to prefrontal lesions but neither writer includes hyperactivity in summarizing frontal lobe symptomatology. Both note that some monkeys became hyperactive while others appeared dull, indifferent or somnolent. Jacobsen (8) is clearly to be credited with recognizing that hyperactivity is one of the principal symptoms of prefrontal destruction in the monkey, 4 of the original series of 5 monkeys were hyperactive and in a later series (9) 2 of 3 monkeys were hyperactive. But only in two recent studies has altered activity been objectively and quantitatively recorded and the lesions made with modern intracranial surgical techniques.

All of Richter and Hines' (21) series of 4 monkeys in which the prefrontal pole or cortex was removed were hyperactive. With respect to localization within the prefrontal cortex, they conclude that activity is particularly controlled through area 9 and also by the striatum. However of the 2 animals in which area 9 was removed bilaterally only one showed really marked increase of activity (4). They further state that unilateral and bilateral ablation of areas 8 or of 10-11-12 had little or no effect on activity. Contrary to this, Kennard and Ectors (10) reported that unilateral or bilateral ablation of area 8 in monkeys definitely produces hyperactivity. Kennard, Spencer and Fountain (11) later concluded that bilateral and separate ablation of

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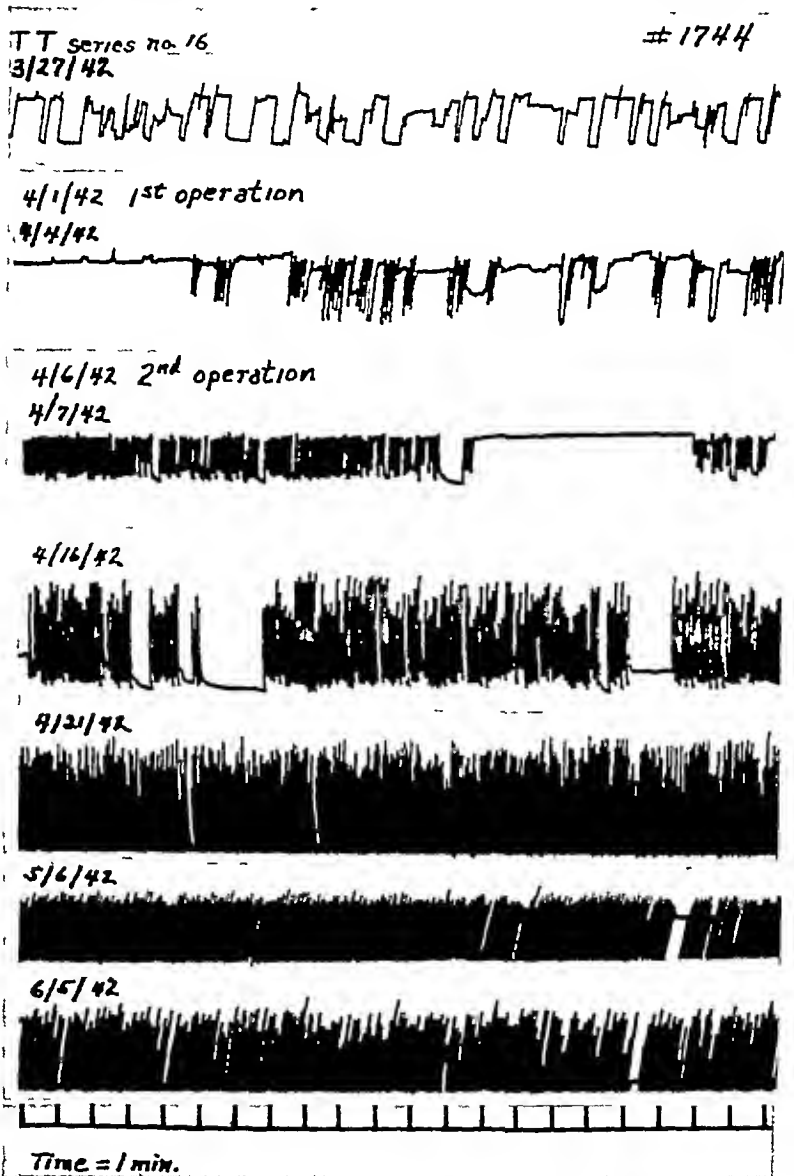


FIG 1 Records showing development of hyperactivity after bilateral ablation of area 13 (second operation) subsequent to ligation of superior longitudinal sinus at its anterior end. Note rapid onset of hyperactivity after second operation and that maximal hyperactivity was reached in two weeks.

the pauses in the records of normal animals. Thus the effect of area 13 lesions is not only to increase the amount of activity but also to simplify the complex interplay of postures and movement.

over a freely movable galvanized iron pan, one end of which rests on a pneumatic pad. The pad is connected with a tambour which records upon a long-paper kymograph in ink. Any movement toward or away from the end resting on the pad causes an excursion of the pen and the height roughly reflects the extent of movement. This apparatus, unlike that of Richter and Hines (20) is only semiquantitative but does record graphically the spatial and temporal pattern of the activity, it is free from the objection of emphasizing a turning pattern of locomotion. Records were made before feeding, usually in the morning, they were of 3 hours' duration, and were obtained on 3 consecutive days before operation. Activity was recorded immediately before operation and on the 1st-5th postoperative days and 2, 4, and 8 weeks postoperatively.

## RESULTS

*Activity* Bilateral ablation of area 13 of the orbital surface of the frontal lobe in one stage has produced marked hyperactivity of immediate onset in 4 consecutive monkeys. The postoperative activity has certain characteristics which make it easily recognizable. It is a change in quality and not merely a quantitative increase of normal spontaneous activity. Thus an operated animal at a time when hyperactivity is minimal is distinguishable from a normal monkey which might display an equal amount of total activity. Of first importance is that the hyperactivity is manifested clearly only in locomotor activity, other forms of movement tending to be restricted, especially in the first postoperative week. Conspicuous is the methodical, stereotyped character of the "pacing" which constitutes the main motor performance of these animals. In a square cage this tends to be about the perimeter of the cage and not consistently in one direction, in a long, narrow cage it is reminiscent of the pacing of a caged lion though much faster except in the first postoperative days. Though the total amount of time spent in walking is greatly increased, the pacing is not strictly incessant for it tends to occur in "bouts" of walking punctuated by rests or pauses. Even when the periods of walking are short and the pauses are long as in the first postoperative days there is a characteristic stereotypy of the operated animals' walking that can easily be seen in the activity records.

The record of a normal monkey (Fig. 1 and 3) reflects a varied pattern of movement about the cage. Rarely is seen more than one or two regularly spaced excursions representing rhythmically repeated "round trips." Periods of activity, periods of slight activity and periods of rest alternate in an infinitely varied pattern. Little evidence of such random activity can be seen in the records or cage activity of area 13 animals. The activities of the operated monkey are largely reduced to two: either methodical walking or quiet resting. The record rarely shows a single translocation from one end of the cage to the other followed by a rest or random activity (denoted by small excursions superimposed on a change in the base line), once walking is initiated it tends to continue at a regular rate for many round trips. It is as though an attempt to move from one end of the cage to the other initiates perseverative walking. Such bouts of walking may last as long as the test period of 3 hours. The pauses too appear not to be interrupted by small random movements (small excursions of the recording pen) that often mark

control the factor of incidental damage to the frontal pole, in a second animal (T T No 21) the frontal pole was removed (area 10 and part of area 11, Walker) after ligation of the sagittal sinus. This failed to produce any hyperactivity either early or late (2 months) after the procedure. The similarity in activity before and after operation is shown in Fig 3. The experiment involving ablation of area 14 described below is further evidence

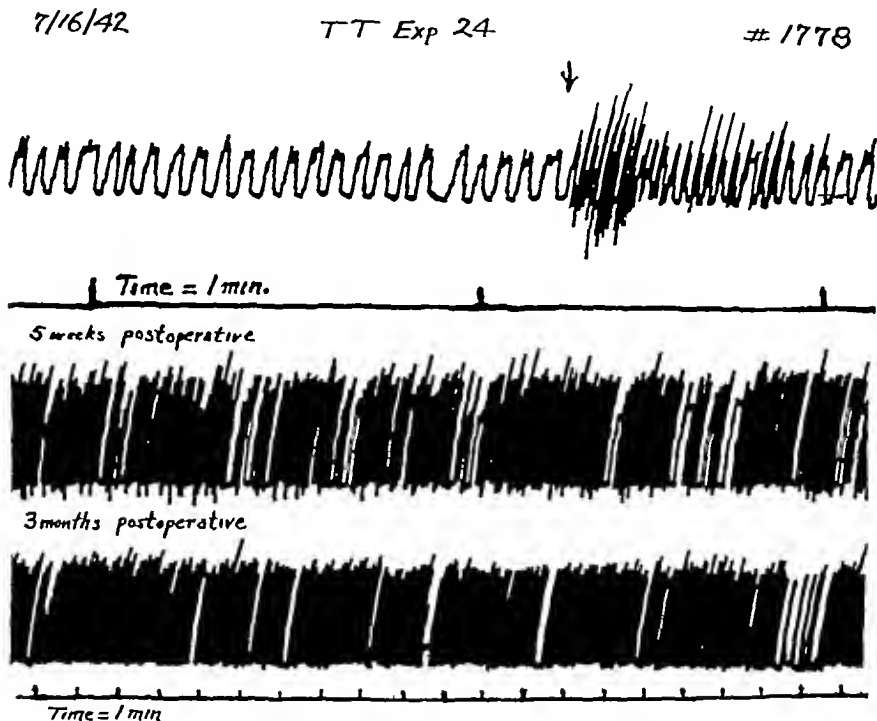


FIG 2 Effect of bilateral area 13 lesion on activity. Upper record, made with a fast drum, shows speed and regularity of pacing. The record after the arrow shows accelerated running in response to presence of the experimenter. The two lower records demonstrate marked hyperactivity in the late postoperative period.

of the same weight. It is unlikely therefore that ligation of the sagittal sinus or the slight damage incidental to elevating the frontal pole would account for the hyperactivity.

*Specificity of area 13 lesions.* Preliminary experiments, too few to allow definite statement, suggest that portions of the orbital surface other than area 13 are without the same effect on hyperactivity. The ablation of the tip of the frontal lobe (area 10 and part of area 11, Walker) mentioned above accounts for the rostral portion of the orbital surface. In another monkey (T T No 23) area 14, which occupies the posterior portion of the internal orbital gyrus (gyrus rectus), was ablated bilaterally. This animal was in

*Onset and persistence of hyperactivity* Hyperactivity has consistently been observed as early as the first postoperative day. At a time when the effects of surgical shock, anesthesia, etc. were still apparent, characteristic pacing was observed, it is slow and frequently interrupted by periods of immobility but does not differ in quality from maximal hyperactivity. A record of activity made on the first postoperative day is shown in Fig. 1. The amount of time devoted to pacing and the rate of walking increases to a maximum in the 3rd or 4th week. In the second postoperative month total activity diminishes somewhat. However, as can be seen in Fig. 2 there may be little or no decrease, and in no animal was there a return to the pre-operative activity level. Two animals (No. 16 and 24) have been kept for more than 6 months and continued to exhibit striking hyperactivity, the alteration in behavior apparently is permanent. The negative symptoms described in the next section, unlike hyperactivity, are most marked in the first and second weeks.

*Negative symptoms* Behavior deficits, at least in the first week after operation, are almost as striking as the overactivity, though difficult to catalogue. There is a definite reduction in emotional expression. Fear seems reduced. Aggressive behavior consisting of opening the mouth with jutting forward of the head and shoulders and fixed gaze so typical of the normal macaque is absent for some days or weeks, and this appears to be permanent in some animals. Unmistakable too is the lack of response to human presence, instead of watching the observer intently as the cage is approached the operated monkeys when not pacing tend to sit gazing into the distance with a blank expression. Later after the operation the animals become more reactive, perhaps over-reactive, to the observer but excitement still produces an abnormal response, which is to accelerate the rate of running (Fig. 2). This seems to take the place of the usual fear or aggressive responses. The distractibility emphasized by other observers could not be identified. At first food tends to be neglected. When offered a piece of fruit the animal reaches for it but allows it to slip through the hand, the empty hand is often brought to the mouth. Or a bite or two is taken and the food then allowed to fall to the floor. Such behavior persists only for a few days after which the animals eat well.

*Controls* The operative technique used involves ligation of the superior longitudinal sinus at its anterior end and some retraction on the tip of the frontal pole. To control the possibility that a generalized prefrontal damage arising from these procedures rather than ablation of area 13 causes the hyperactivity, the following experiments were performed. In one animal (T.T. No. 16) a "dummy" operation consisted of the usual procedures to and including ligation of the longitudinal sinus and severance of the falx. This appears from the activity record (Fig. 1) to have increased activity slightly but neither hyperactivity nor any other departure from the normal was detected in cage behavior. In two additional experiments the sinus was ligated as a part of other procedures without any sign of hyperactivity. To

expressed in Calories per kg body weight. When averaged for a period of 5-10 days this value was found to be quite consistent from animal to animal. A comparison of pre- and postoperative food intake indicated a slight increase for two animals and a slight decrease for the third (See Table 1). In view of the metabolic cost of the hyperactive state, which is also reflected in a slight weight loss, no significance can be attached to these slight changes. Ablation of area 13 certainly does not appear to cause true hyperphagia, the same result was obtained for prefrontal lobectomies by Kennard, Spencer and Fountain (11).

Since area 13, according to the stimulation experiments of Bailey and Sweet (2) is related to gastric tonus, and since the frontal lobe has been re-

*Table 1 Food intake and body weight*

Experiment No		Preoperative	Postoperative
14	Av C/day	306 00	366 00
	Body wt	3 25	2 85
	C/kg	94 00	128 00
16	Av C/day	351 00	380 00
	Body wt	3 25	3 00
	C/kg	108 00	127 00
13	Av C/day	310 00	291 00
	Body wt	2 50	2 32
	C/kg	124 00	125 00

lated to gastrointestinal motility by Watts and Fulton (24), carmine tests were conducted on 3 animals. There was no evidence of increased rate of transit of carmine, which is in agreement with the findings of Kennard, Spencer and Fountain (11) for other prefrontal lesions.

Resting rate of oxygen consumption was not increased in 2 area 13 animals. These observations will be reported in full by Dr M A Kennard.

#### DISCUSSION

The hyperactivity produced by ablation of area 13 of Walker appears to be qualitatively identical with the behavior described as hyperactivity by previous workers. An important characteristic of the phenomenon which is not conveyed by the conventional terms "hyperactivity" or increased "random" or "spontaneous" activity is that the phenomenon is manifested chiefly in the sphere of locomotion. No hyperactivity of membral movements, of eye and head movements, of facial expression or in the "posturing" of the animals could be detected. On the contrary activity in other than the locomotory sphere appears to be reduced. Certainly the infinitely varied play of postures as well as emotional expression normal to the macaque is largely lost. Quiescence rather than agitation characterizes the cage be-

excellent condition for 5 days when it suffered a head trauma resulting in a subdural hematoma. During the first 5 days, careful observation revealed no similarity to the area 13 animals which all became definitely hyperactive within this period. At no time did he pace, exhibit diminished emotional behavior or ignore the observer. The animal's cage activity could not be distinguished from that of a normal macaque in an adjacent cage. The lateral region of the orbital surface has not been explored. From this limited expe-

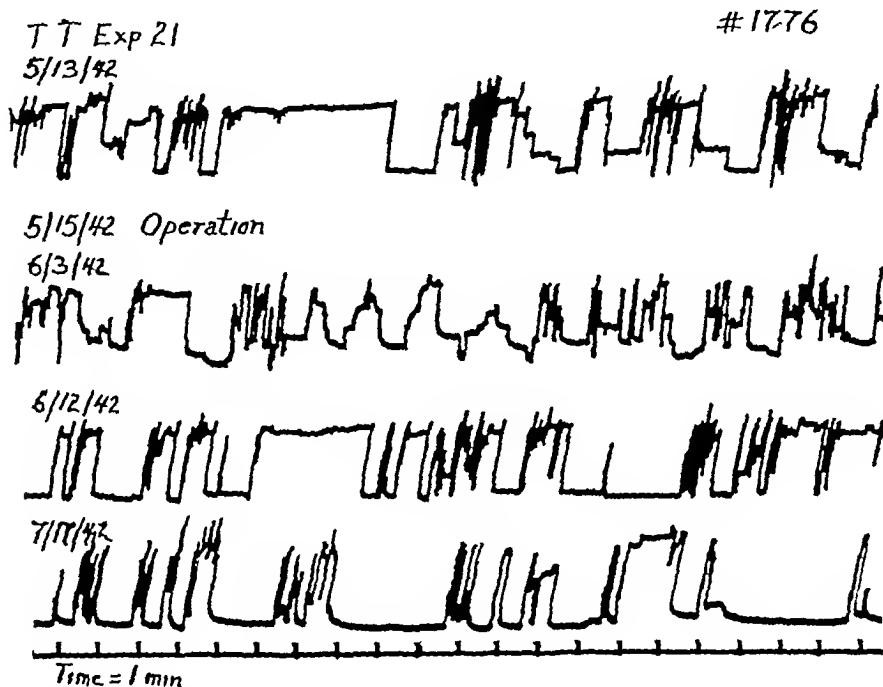


FIG 3 Activity records showing normal activity after ablation of tip of frontal pole with ligation of the superior longitudinal sinus at its anterior end. Hyperactivity is absent both as an immediate and a late event after operation.

rience no suggestion is forthcoming that regions on the orbital surface other than area 13 are concerned to any large degree with hyperactivity.

*Food intake, gastrointestinal motility and oxygen consumption* Somewhat the same ambiguity exists with respect to hyperphagia as a symptom of frontal lobe lesion as for hyperactivity, it has been found in the rat (20) but not in the monkey (11). Since afferent and efferent visceral connections of the orbital region have been demonstrated physiologically (1, 2) the food intake was measured before and after ablation of this region. In three area 13 animals the *ad libitum* food intake was determined for a standard diet made up of foods from the laboratory diet. Carrots, potatoes and bananas were fed in fixed amounts and peanuts and bread were allowed in unlimited quantities, the daily residues were sorted and weighed. Food intake was

Consistent with the rapidity of onset and development is the excessive degree of hyperactivity induced by this relatively small ablation. Within two weeks of operation the hyperactivity appears to approach that reported for large prefrontal lesions, though exact quantitative comparison is difficult. One of the criteria of maximal hyperactivity, continuous running for periods of an hour or more, is met by area 13 animals. The standard drum speed is too slow to allow exact calculation of the rate of running in all records, however, for one animal it is 20 round trips per min in a cage 4 ft long. A record made with a fast drum is shown in Fig. 2. While the hyperactivity of area 13 animals appears to exceed that of prefrontal lobe animals in the first weeks after operation, the phenomenon may be more persistent in the latter. Finally, as pointed out in the review of literature, large as well as small prefrontal lesions unaccountably fail on occasion to induce hyperactivity. It has occurred consistently in a short series of 4 area 13 animals.

There are then three indications that area 13 is an important area for the control of activity, hyperactivity produced by ablation of this area approaches in magnitude that from large prefrontal lesions, it sets on more quickly after operation, and it seems to be less variable in occurrence, degree and time of onset. The critical area appears to be area 13 and not the whole orbital surface. At least, two bordering areas, 14 and 10 plus a part of 11, have been extirpated as a primary procedure without producing any sign of hyperactivity of early onset. On the whole, the degree and rapidity of onset of hyperactivity induced by ablation of area 13 would seem sufficiently pronounced to preclude its being due to damage to other prefrontal areas.

The existence on the orbital surface of an unsuspected area related to activity raises the question whether it has been included in lesions designed to destroy all prefrontal tissue. Area 13 occupies the most posterior portion of the orbital gyrus (also called the posterior orbital gyrus, 2). It is not unlikely that in attempting a prefrontal lobectomy and especially a lesion of areas 9-12, area 13 may be included or excluded from the block by deflecting the ablating instrument either slightly anteriorly or posteriorly with relation to the frontal plane. Any variation from lesion to lesion at the orbital surface might contribute to the variability in degree of activity produced by large prefrontal lesions. Furthermore area 13 is so situated that it is likely to be spared by prefrontal ablations designed to preserve the striatum, and ablations designed to include the striatum are likely to include area 13 as well. The converse of the latter is also true. However, intense hyperactivity can result from a lesion (Fig. 5) which spares the caudate and damages only slightly the inferior border of the putamen. No cellular reactions suggestive of ischemia of the striatum were discovered. Mettler and Mettler (18) implicate the striatum in the production of a phenomenon termed "forced, cursive hyperkinesis," the relationship of which to the hyperactivity described above is not clear.

It has been argued (17) that hyperactivity from prefrontal lesions is of delayed onset and that from striatal lesions is of immediate onset.

havior when not pacing. In short, the release of activity appears to be fairly specific and not a generalized increase of activity. The early view that the frontal lobes exert some *general* "inhibitory" effect on cortical or subcortical centers is therefore untenable.

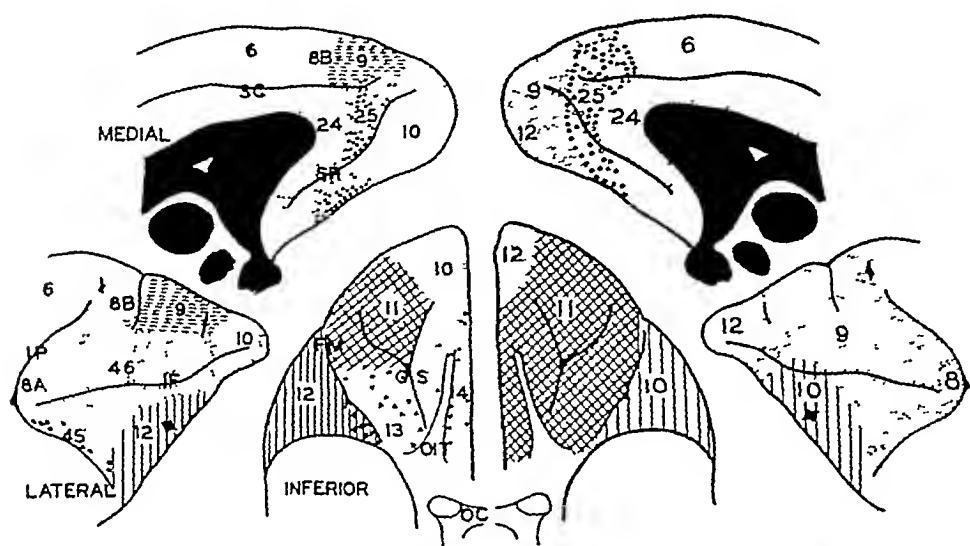


FIG. 4. A. E. Walker's cytoarchitectural map of the prefrontal lobe of *Macaca mulatta* (left) compared with Brodmann's map of *Cercopithecus* (right). The numerical designations in Walker's map are derived from Brodmann's designations of comparable areas in the human cerebral cortex and hence do not necessarily have anything in common with Brodmann and Vogt's designations for the monkey.

A feature of the hyperactivity induced by lesions of area 13 which indicates that this region is especially associated with the control of activity is the rapidity of its onset after operation. In all monkeys characteristic pacing was observed on the morning of the first postoperative day. Objective records of activity showing typical bouts of walking were obtained on the first day in the apparatus, which was 1 to 3 days after operation. They differ from later records in the length and frequency of pauses and in the rate of walking but the bouts of walking are of the characteristic methodical, stereotyped pattern. An early onset of hyperactivity seems to be unique to area 13 lesions. Kennard *et al.* (11), for example, state that "The hyperactivity is usually preceded by a period of hypoactivity immediately following the operation which may last a few days or weeks. During this time, the animals appear confused, lethargic, slow and difficult to arouse." Their animals for the most part had fairly large lesions of the prefrontal area. Richter and Hines (21) also noted that the onset of hyperactivity was delayed (e.g., 20 days after bilateral lesion of area 9), when lesions were limited to the cortex of the frontal association areas, while the onset was more precipitous after striatal lesions.



certain ill-defined behavior changes All of these are most marked in the first postoperative week

5 Hyperactivity is accompanied by a weight loss and only a slight increase in food intake

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so that the thalamus rather than the cortex controls activity through the striatum. Since area 13 lesions which do not damage the caudate nucleus (Fig 5) produce immediate hyperactivity this argument loses weight and leaves open the possibility of an area 13-striatal connection for the control of activity.

#### SUMMARY AND CONCLUSIONS

The posterior portion of the external orbital gyrus (posterior orbital gyrus), which Walker has recently differentiated as a new cytoarchitectural

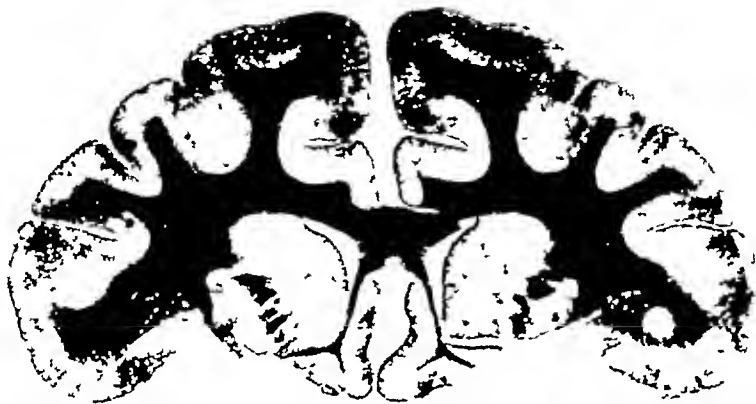


FIG 5 Frontal section through the brain of a monkey made hyperactive by ablation of the posterior orbital gyrus of both hemispheres. The ablation extends to the inferior border of the striatum which is only slightly damaged. Expt T T no 13.

area (area 13) and which Bailey, Bremer and Sweet (1, 2) have demarcated physiologically from adjoining areas, has been ablated in a series of monkeys. This procedure produces in a marked degree many of the symptoms that have been described for prefrontal lobectomy by various workers under the term hyperactivity. The results of area 13 lesions are as follows:

1. Hyperactivity is manifested by long continued, methodical pacing or running of a regular, stereotyped character.
2. Hyperactivity from area 13 lesions is quantitatively great, is consistently obtained and is always manifested in some degree within the first or second postoperative day, whereas similar hyperactivity from other prefrontal areas is said to be delayed in onset (as long as 2-3 weeks) and does not invariably occur.
3. Ablation of neighboring regions by the same operative approach was without effect on activity.
4. Other motor activities are not marked by hyperactivity but rather suffer reduction. Random, spontaneous activities and posturings are reduced in variety and quantity, as is emotional expressivity. There are also

wrapped in tinfoil *D* is a small neon tube in the handle of the needle holder that carries the stimulating needle *E*. When the latter approximates the skin to about 0.5 mm, the lower plate of *C* discharges through the needle and air gap to ground potential with a flash signal in *D*. If resistances *A* and *B* are too high to permit a steady flow of current across the gap, the discharge is a single brief shock, and the needle can be raised again before the condenser is recharged through *B*. At lower settings of the resistances a repetitive discharge is permitted at a frequency determined by the values of capacity and resistance selected.

The three types of endings revealed by the Von Frey hair technique and its variants are readily identified by electrical stimulation of single endings, those mediating ordinary touch or light pressure, those for touch associated with hair shafts, and those inducing the sensation to be identified as prick, which becomes pricking pain on a stronger stimulation. Occasional spots sensitive to cold have been encountered which also responded to electrical stimulation, but none for warmth, these two have not been specifically explored for.

#### RESPONSES TO SINGLE STIMULI

On regions of skin where sense organs are relatively far apart, prick and touch can be readily differentiated by spark stimulation. One suitable location is the dorsum of the web between the fingers. Here prick has a much lower threshold than touch to electrical stimulation of single endings. This is also true for the back of the hand and arm generally, although the opposite is true of the skin covering the balls of the fingers. The difference is in part assignable to differences in depth of endings below the surface, for if the skin of the ball of the finger is sandpapered sufficiently the order is reversed, the prick threshold becoming equal to or lower than that for touch as is found elsewhere. The extreme sensitivity of the fingertips to touch may be due to the greater number of receptors there rather than to the great sensitivity of individual receptors, in the case of mechanical as well as electrical stimulation, for as will be indicated below, the activities of adjacent touch receptors are strongly summated subjectively.

A single stimulus applied to a single prick spot at the threshold for subjective identification is not painful, but elicits a tactile experience usually accompanied by a faint aura of itch. The latter is especially noticeable if the stimulus is repeated immediately. This tactile sensation from a single receptor is so faint as to be recognized only when attention is closely paid to it. It has a long latency, and is not associated with a feeling of pressure. As the stimulus is increased, a stinging or pricking sensation is induced, with a rapidly shortening latency and an increasing after-effect of prick which often fades to itch. A single strong stimulus has an effect which is sharp, stinging and persistent. On the other hand a single threshold stimulus applied to a touch ending is experienced as a slight tap. As the stimulus is increased, the tap feels heavier, but does not persist. The latency of the

# RESPONSES TO ELECTRICAL STIMULATION OF SINGLE SENSORY UNITS OF SKIN

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## INTRODUCTION

IN SPITE of numerous and elaborate studies on skin sensibility, the cutaneous receptors have yet to be adequately investigated by one of the useful methods of neurophysiological analysis, that of direct electrical stimulation by single and repeated shocks. Although this is not the normal means of activating such receptors, the handicap here is no greater than where this method is applied to the study of nerve trunks or nuclei of the central nervous system. In fact the use of artificial or unaccustomed methods of approach has the advantage of minimizing the prejudices that arise from customary experience. Certain elementary questions still remain undecided after extensive study of sensory receptors. The receptors which serve the different modalities of sensation are still in dispute, and the earlier inference that pain endings were more superficially located in the skin than touch endings has recently been challenged (14, 15). The very character of the excitation process in the receptor is unknown. Whether a persistent excitation of the receptor induces repetitive excitation of its nerve fiber as a constant current does, or whether the sense organ itself responds repetitively, remain equally problematical.

To approach some of these problems a method was sought for effectively stimulating single sensory receptors without mechanically deforming the skin. A small electric spark was employed, and the device of Fig. 1 was used for preliminary exploration of sensory receptors in human subjects. The sensory experiences reported were taken as data. These data, combined with what is known about nerve responses to various forms of sense organ stimulation in animal experiments, allow certain inferences to be drawn as to the physiological behavior of the sense organs concerned. The observations hereafter reported may call for further checks on animals under similar conditions of stimulation.

In Fig. 1, direct current at 3000 V potential from a filter circuit charges the whole stimulating structure through a protective resistance. A and B are carbon line variable resistors of several hundred megohms each, C is a variable condenser consisting of concentric test-tubes

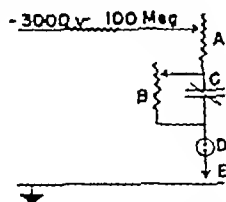


FIG. 1 Diagram of simple apparatus for stimulation of single sensory endings without mechanical contact. See text.

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side maintaining a constant condition at the gap, the direct ionizing circuit through *H* also acts with the circuit through *F* as a voltage-doubling device, permitting the use of a small condenser and brief time constant of the arc discharge

The operation of the device is rendered audible in ear phones at *I*, one of which is connected in each of the two parallel circuits, through the body and through the shunt, a click being delivered to one ear when a stimulus passes through the skin, otherwise recording only in the other ear. The stimulus cycle can be photographed on the oscillograph *O*, whose sweep is timed with *K*. A tap key *N* signals on the oscillograph the approximate latency of the subjective sensation. Alternately *H* can be thrown to deliver only constant current to the circuit beyond the gap *G*, or at open position a constant (or high frequency) arc discharge occurs across *G*. The technical details of this apparatus are given in the appendix below, together with certain physical considerations involved in the operation of an arc at low current values.

With the body grounded through the recording circuit, stimuli are applied through a fine wire in a needle holder, the contact of the wire itself being too light to be felt, or a spark will jump to the skin if the wire is held under a hand lens near the skin. Alternately

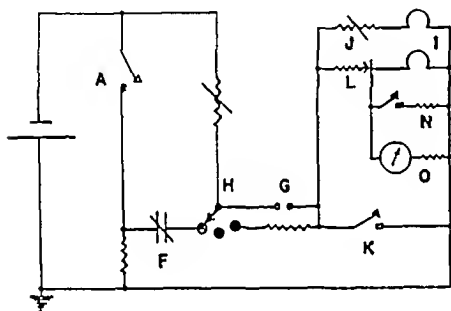


FIG 2 Schematic diagram of apparatus for delivering repetitive electrical stimuli to a single sensory ending. Description in text, and further details of construction and operation in Fig 6

we have employed thin mica disks 1 cm. in diameter, with a fine central perforation over which a 2 mm. disk of foil was laid, and the whole compressed so that the foil extrudes through the perforation to make contact with the underlying skin. Such disks contain 1 mg. of mica and 2 mg. of foil, can be dropped on the skin without being felt, and are fixed in place by smearing the edges with vaseline. Contamination of the dry skin with vaseline seems to offer no impediment to the current, and may facilitate electrical contact, as it does on metal surfaces generally. Moistening the skin surface with salt solution or distilled water on the contrary increases the effective threshold for stimulation, probably by diffusing the current above the highly resistant cuticle.

If a repetitively discharging needle is drawn slowly across the dry skin, under observation through a binocular, apparent prick loci, that is points of strongest sensation, are most frequently encountered in the depressions between polygonal areas of mechanically stiffer cuticle. It is probable therefore that one of the chief factors determining the thresholds of single sense organs of the skin for electrical stimulation, and to some extent for mechanical stimulation, is the structure of the skin itself and its mosaic of electrically and mechanically resistant areas.

*Responses to repetitive stimuli.* There has been considerable disagreement in the literature concerning the quality of sensation with relation to intensity of stimulation. The question is usually presented in the form: Do all receptors mediate pain when sufficiently stimulated, or is pain a single modality mediated by its specific sense organ? In a previous paper (9) we concluded the latter, with the reservation that a single stimulus to a few nerve fibers passing to potential pain endings caused only tactile sensation. It is perhaps academic to argue whether an incipiently painful sensation

sensory experience is far shorter than that for threshold prick. Strong single stimuli become unpleasant, and as they do so pain may be felt, but exploration of territory adjoining the receptor with a stimulus of constant strength usually reveals a sharp focus of this painful sensation at a nearby prick spot, with a steady increase of prick sensation in passing from the previously identified touch spot to its neighboring prick spot. The prick resulting from strong electrical stimulation of an isolated touch spot is therefore in the larger part or perhaps wholly due to spread of current to adjacent lower-threshold prick endings. Over the forearm few pure touch areas can be found although one encounters with a weak stimulus numerous foci of pure prick without accompanying sensations of touch. The reason for these relations is that the electrical threshold for prick is in general considerably lower than for touch. Touch can be recognized with stronger stimuli as a feeling of abrupt contact or tapping against a background of pricking pain.

The thresholds referred to are those eliciting sensory experience, and are not demonstrably the thresholds for initial activation of a nerve fiber by its receptor. Since the nerve fiber's impulse is all-or-none, if one impulse in one nerve fiber resulted in a definite sensation, a slight increase above its threshold should at least double the sensory effect experienced, and this should be recognizable as a step-like increase in sensation. No such unit augmentation is recognizable, even when experienced from touch spots where the sensation at apparent threshold is rather definite. Therefore it is inferred that even threshold *sensation* experienced from the stimulation of *one receptor* involves the repetitive response of the nerve fiber leading from that receptor, and as the number of nerve fiber responses increases with increase of the stimulus, the sensation increases by fractions too small to be recognized as other than a smooth gradation.

The third group of receptors to be considered here, touch organs at the bases of hair shafts, are more similar in their responses to touch organs generally than to prick endings, but are not identical to these. They will be considered more fully below. The sensation induced by a single stimulus to a hair shaft receptor is indistinguishable from that induced by bending a hair mechanically. It is not experienced as a tap or light blow, as is the stimulus to other touch receptors.

#### PERSISTENT AND REPETITIVE EXCITATION OF SINGLE SKIN RECEPTORS

*Technique.* For repetitive stimulation the simple apparatus noted above was not subject to precise control, since the value of the stimulus varied with the distance across the gap, and with repetitive discharges the first stimulus was greater than the succeeding. There is also a lower limit to the energy that can be delivered across such an air gap, and this limiting value proved to be above threshold for many sense endings. The apparatus of Fig. 2 was therefore devised for more accurate and flexible manipulation. A constant high voltage is applied through a high resistance vacuum tube and switch *H* to maintain ionization of the air gap *G*. Pulses from the same source are applied to the variable condenser *F* and thence to *G*, by means of an electronic tripping device at *A*. The intermittent shocks discharged across *G* stimulate the skin at *L*, during an interval that can be limited by the short-circuit *K*, and at a strength regulated by *F* and also by the shunt circuit at *J*. Be-

when pulled from their moorings, but the threshold for pain even to gross pulling of single hairs is very variable. The sudden jerk with which hairs are most expeditiously removed is sometimes not painful, but always leaves an after-effect of itch, (see below). A few hair endings have been found where the threshold for prick was at least ten times that for touch.

In connection with hair-organs, the following observation is perhaps significant. If a single hair shaft is poked with a fine instrument, the hair must be very considerably bent before any sensation is induced. If now a hirsute area is disturbed so lightly, even with a breath of air, that any single hair is bent less than its threshold for recognition requires, the sensation is unmistakably more intense. Aside from the obvious demonstration of spatial summation, this observation indicates that the threshold for subjective sensation to sense organ activation is materially higher than the threshold for response of the nerve fiber itself, in other words, more than one nerve impulse from one sense organ is required for a sensory effect. This is analogous to the repeated finding in direct stimulation of nerves leading to central nervous system responses as recorded electrically, more than one impulse over one nerve fiber is often required to fire a central synapse.

*Distributions of sensory endings as tested by electrical stimulation.* A stimulus pattern was chosen by trial which gave the most clear-cut differences in sensation from prick, touch, and hair-shaft endings respectively. This proved to be a continuously repetitive sequence at about 5 per second, varied in intensity for different types of ending necessarily, but held constant otherwise. The needle was raised from the skin between trials, which consisted each of about 1 second contact. The subject was the present writer, normal so far as indicated by clinical skin tests. He had the cues of sound in the ear phones, vision of the needle through a X2 hand lens, and the oscillographic trace of the stimulus record when significant. Areas were explored at three locations along the lower arm, on the back of the hand, and on the nail bed, balls of fingers, and palmar surfaces of phalanges.

*Prick receptors.* Stimuli which individually caused slight prick at the most sensitive points showed summation at 5 per sec. At this strength the spots were sharply localized, and moving the needle as little as 1/10 mm was sufficient to abolish all sensation. With increase of strength these points expanded, and other points were found, the intensity of sensation decreasing with distance from such focal points. With stimuli strong enough to induce light pressure sensations, prick could be elicited from almost any area, but with striking differences in intensity, varying between burning pain and slight prick. Drawing the needle across the skin at uniform rate gave the feeling of irregular jumpy movement, with occasional sharp jabs as if the point had suddenly caught and penetrated the skin. Distances from one prick focus to another varied, from 2 to 10 mm on the arm and hand, (Fig 3) but due to poor subjective localization below threshold for touch, it is difficult to decide whether two adjacent spots are referred to the same locus or not. Consequently it cannot be judged whether one nerve fiber supplies

shall be called painful in anticipation, or tactile in retrospect, since it is naively obvious that a sensation is painful only if it is painful *enough*. There is however distinct qualitative difference between even threshold sensations from stimulation of regions that on stronger stimulation elicit respectively stinging or pricking pain and superficial touch or light pressure.

Stimulation of what will hereafter be designated touch receptors, with weak single shocks, gives the impression of a light blow, and this sensation of abruptly applied pressure is only intensified on moderate increase of intensity. It is simply repetitive at low frequencies, with slight temporal summation. At higher frequencies the sensation becomes smooth pressure. At higher intensities it becomes unpleasant, especially at low frequencies, and is usually contaminated with pricking pain due to spread of current to adjacent prick endings. Usually where touch is at a maximum for a given intensity of stimulus, prick is at a minimum and vice versa.

In contrast, repetitive stimulation of what will be designated hereafter as prick receptors, even stimulated at a threshold where a single stimulus is not painful, rapidly summates to frank pain, without any of the feeling that a blow has been struck. In fact a distinctive characteristic of weak repetitive stimulation is the gradual onset of this sensation, doubtless related to the long latency to weak stimuli. It is impossible to obtain from prick receptors alone the vibratory character of the sensation so readily elicited from touch receptors.

The tactile organs at the bases of hair shafts lie in some respects between these two. The latter organs, (which will be designated hereafter as hair-receptors, in spite of the fact that other receptors often occur here,) are difficult to stimulate through the skin surface, probably because they lie deep at the root of the hair-shaft. They are readily stimulated by a high voltage shock led to the proximal 1 to 2 mm. of the hair above the skin. By this circumstance they are reliably differentiated from other touch organs under electrical stimulation. Single weak stimuli give a sensation identical to that caused by slight bending of the hair shaft. Repetitive stimulation results in summation to a steady sensation at considerably lower frequencies than that characteristic of touch organs, but they show less summation than do prick endings. They do not give the sensation of a tap on the skin surface, as do touch organs, they rather give the feeling of something rough drawn across the hair. Strong stimulation via hair shafts always results in pain, and from some hairs only pain can be recognized, the pain then masking the tactile sensation if a hair-receptor is indeed present. On the other hand, a few hair shafts have only ordinary skin touch in their vicinity.

The fact that one finds such a varying relation between the thresholds for prick and for tactile sense associated with hair shafts indicates that the two sensations must be mediated by different organs, lying at varying distances from the hair shaft. Whether the hair tactile organs themselves give pain on strong stimulation cannot be finally decided under these circumstances. We have not been able to find any hairs that failed to cause pain



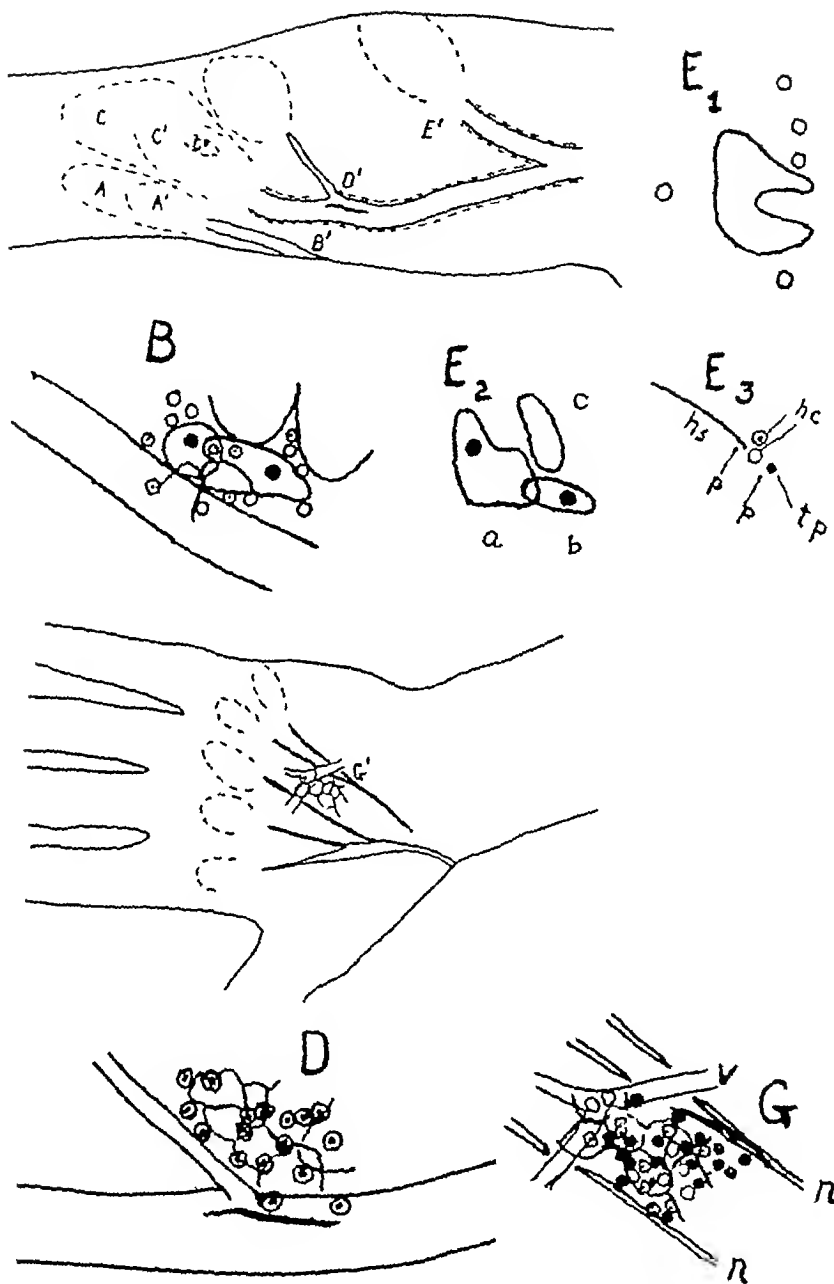


FIG 3 Diagram of dorsal surface of right forearm, and of the right hand,  $\frac{1}{2}$  size, to illustrate effects of electrical stimulation of nerves and of sense organs in and below the skin. All stimuli involved a 2300 V source, employing various capacities at *F* in Fig 2. Pain was reached at the most sensitive skin points with the variable capacity of 0.0001 Mf set at about  $\frac{1}{2}$  of this maximum value. Employing 0.001 Mf, nerve trunks and branches could be stimulated over wide paths as outlined in full lines on forearm outline, and a sensation of

more than one prick focus, although a unit field almost certainly includes an area around each focus

Such a distribution of excitability for prick might be due to several causes, to varying depths of sensory endings below the skin; to variations in skin resistance, or to variations in intrinsic excitability between end branches of one axone. That one factor is either variation of skin resistance or of depth below surface is indicated by the fact noted previously that many "high" points for prick lie in minute grooves between more stiff areas of skin. Also, if the skin is shaved closely, or the epidermis removed with sandpaper, the electrical threshold is materially lowered. In such an area, a scratch by a grain of sand through the epidermis, even too slight to cause irritation, has a greatly lowered threshold to prick. That is, one can produce a "high" spot by removing the cuticle locally, and normal variations in skin resistance will therefore be a factor in such localization.

*Touch receptors.* Skin shaved a few days previously will contain stubs of hair shafts long enough to manipulate but too short to interfere with exploration for touch. The spatial unit for the latter sense appears to be a relatively uniform area rather than a high point grading off to relative insensitivity. These areas are a centimeter or more in diameter toward the elbow, grading to a few millimeters on the back of the hand, and one or two millimeters on the ball of the finger (Fig. 3). Usually, but not always, each area will have one point of definitely greater sensitivity to a given strength of stimulus. This can be located within less than half a millimeter, with no obvious relation to irregularity of the skin over it (Fig. 3 E). Subjectively, the sensation at that point is stronger, and at least while watching the needle, stimulation of other parts of the area seem to be referred to this point. Above threshold, stimulation at any point within an area feels the same, except at the high point, that is, there is not the progressive gradation from peaks to valleys found associated with prick foci. The margins of adjacent areas may overlap, or there may be narrow spaces between them without touch sensation unless the stimulus is very strong (Fig. 3 B). On hirsute surfaces many of the hair shafts are situated at the peripheries of touch areas, some areas lying entirely between hairs. An exception was noted on the back of the hand, and the hair endings here gave a sensation when stimulated that could not be distinguished from ordinary touch, which in fact was probably also stimulated. The margins of these areas are located by drawing the needle slowly across the skin, marking points where the sensation shifts abruptly in subjective localization. It appears obvious that these are the endings involved in compass-point tests, of which the above maneuver is a modification, though it cannot be said that each area is served by one fiber.

In Fig. 3 tracings are presented of groups of touch areas. It will be noted that the high points for prick show no correlation with touch areas, and there seem to be more prick spots than touch areas, at least over the forearm and hand. On the balls of the fingers this is not obviously the case, but here the

needle is employed, than with electrical stimulation. Across the ball of the finger a moving mechanical contact gives an apparently smoothly changing localization, as contrasted to the step-like progress with electrical stimulation. This may be assigned to the gradual deformation of the skin ahead of the mechanical stimulus.

*Hair receptors* The distribution of sense receptors associated with hair shafts appears to be one to each hair, or if there are more than one they must lie very close about the follicle. The sensation cannot be localized subjectively with sufficient precision to judge whether one fiber distributes to more than one hair, but this can certainly not be excluded. The point on the skin which, stimulated electrically, gives a sensation identical with moving a hair, lies usually one fourth to one half millimeter from the hair shaft's point of emergence, in the direction of the hair's slant under the surface, and when an occasional case is found where the sensation is not contaminated by either prick or light touch, this point can be located with the needle very precisely. Not all hair follicles however have a characteristic modality. A few have no sensory points in their immediate vicinity, some give only prick, even when a spark is sent along the shaft itself, and some have only ordinary touch near them. For a few, no specific sense organ could be found electrically, but bending still produced a sensation, perhaps from a sense organ too deep in the skin to be reached by other than currents strong enough to confuse the issue.

*Functional correlation with histological structure* Such rather distinctive distributions of the excitable areas for different senses obviously suggests the possibility of correlating sensation with known histological structure, but it must be recognized that to the many pitfalls which have complicated such attempts in the past, the method of electrical stimulation adds its appropriate quota. Beside the considerations already mentioned of variations in skin resistance and depth of endings below the surface, the question arises what is being stimulated, nerve fibers, their fine end-ramifications, or specialized endings, or the non-nervous enveloping tissues of sense organs.

The specialized structure of at least some sense organs, consisting of non-nervous capsules surrounding the modified ending of a nerve fiber, obviously suggests the function of transforming one kind of energy—that of the external stimulus, mechanical deformation or temperature change for instance which corresponds to the receptor's modality—to another kind, whatever that may be, which is peculiarly suitable for setting up impulses in nerve fibers. The fact that even the briefest stimulus, a sudden prick or an electric shock, can set up a persisting sensation, which must involve a continuing train of discrete nerve impulses in a nerve fiber, strongly suggests that at some site in the sense organ a persisting chemical or physicochemical effect has been induced, which is not all-or-none, and which serves as the intermediate stimulus to the nerve. In fact since the "excitable" part of the sense organ is presumably its nervous part, we might differentiate this

density of distribution of both appears to be greater. The needle, discharging repetitively and drawn slowly across the ball of the finger, *seems* to change its position in a saltatory manner about every two millimeters or less, and these apparent shifts of position presumably indicate the margins between the smaller adjacent touch areas in this region. Prick here requires a much stronger stimulus than does touch, and its threshold is so greatly reduced by removing the cuticle, that the number of separate prick spots is impossible to estimate. On the region just proximal to the ball of a finger, prick again has the lower electrical threshold, and the distribution of prick points is not greatly different from that over the arm.

Foci of touch and prick located by electrical stimulation were checked by pricking with a sharp needle. These electrical high spots for prick coincided exactly with the points where minimum pressure was required for mechanical prick. When a touch area had a similar high focus, this was also more sensitive to light touch than its immediate surroundings. Otherwise there seems to be more variation in touch threshold when the mechanical

touch was referred to the areas enclosed in dash lines. At 0 0011 Mf the paths were broader, indicated by dash lines parallel to the full lines. At 0 0002 Mf the only region where a nerve trunk could be stimulated was along the heavy line below  $D'$ , where the nerve presumably lay closest to the surface. The nerves in hand outline were located with 0 0005 Mf stimulating capacity. The widening of a pathway presumably indicates a shallower depth of the nerve. Crossing above the nerve branches at  $G$  is a vein  $v$  through which the nerves could not be stimulated.

The areas of reference seemed to contract as the nerve stimulus was made weaker,  $A$  and  $C$  correspond to the strongest stimuli,  $A'$  and  $C'$  to stimuli near the edges of the paths, and  $C''$  to the threshold response from along the line below  $D'$ .

$B$ , unit touch areas outlined with a 0 0001 Mf stimulus, drawn actual size.  $\circ$  indicates hair shaft,  $\bullet$  is the high spot for touch in its area. Three of these areas overlap with a fourth, while between the right hand one of these and the area above, a space is vacant for touch. In this space three hair shafts could be investigated for hair-sense uncomplicated by ordinary touch. Located at  $B'$  on outline of arm.

$D$ , touch areas near a nerve trunk, at  $D'$  on outline, 0 0003 Mf stimulus employed. 0 001 Mf gave no referred responses here except along nerve paths as outlined. Hair shafts indicated by circles.

$E$ , three details near location  $E'$ .  $E_1$  a single touch area containing no hair shafts.  $E$ , three adjacent touch areas of decidedly different affect with the same stimulus.  $a$  gave a very strong abrupt sensation, with the spot marked by a black circle even more sensitive, to which any stimulus in this area seemed to be referred. The sensation from  $b$  was less pronounced, but it also had a focal point of reference. From  $c$  the sensation was weak and poorly localized.  $E_2$ , an area about a single isolated hair shaft,  $pp$  are pain foci,  $hs$  the hair shaft,  $hc$  two separate hair endings, one immediately at its base, one further away and at one side.  $tp$  a touch area high spot, where a pain ending adjacent to it was stimulated at about threshold for touch. Over the pain ending itself the threshold for pain was much lower and the location of the pain spots could be confirmed by mechanical stimulation with a sharp needle. The proximity of the other pain spot to its nearest hair ending made it impossible to obtain hair touch without pain, but this could be induced at the more distant hair ending.

$G$  area on back of hand between nerve branches, located at  $G'$  on outline of hand. Touch areas are outlined, circles are hair shafts, dots are high points for pain. Stimulus 0 0001 Mf for pain spots. 0 0003 Mf for touch. There seems to be no correlation between the distributions of touch, pain and hair shafts.

the argument presented above that specialized endings rather than ordinary nerve fibers are stimulated, except that they do mention varicosities, etc along the terminal branches. It may be suggested that such minor modifications may serve as the seat of the physiological specialization which the results of electrical stimulation seem to demand.

#### CERTAIN THEORETICAL CONSIDERATIONS, FORM OF SENSORY MESSAGE IN TERMS OF NEURONE RESPONSES

Whatever the validity of the foregoing inferences, it is obvious that the immediate result of a single stimulus to a skin receptor, however brief, is a single or repetitive response in a fiber leading from it. Differences in sensation due to differences in strength of single stimuli must involve differences in number and frequency of such impulses. In fact, any sensory affect, at the peripheral nerve level, must be mediated by means of specific and all-or-none impulses in the nerve fibers from sense organs. Further, the physiology of the central phenomena evoked must be dealt with in terms of such impulses impinging on central synapses. It will be convenient at this point to evaluate the types of activity induced by our procedures in terms of nerve impulses.

The impulses in a peripheral nerve fiber are variable in two ways only: first, in number, and second, in frequency. The lower limit of response of one nerve fiber to stimulation of its sense organ is one nerve impulse, and this probably fails to reach the higher centers unaided. Various experimental evidence from work on animals (1, 12, 8, 19) indicates what the further stimulation of a sense organ above its nerve threshold means in terms of nerve impulses in its fiber. A stronger stimulus excites a sense organ more intensely, and its excitation will persist for a longer time. The intensity of excitation appears in terms of frequency of discharge of impulses in the nerve fiber, its persistence in terms of numbers of discharges. To a first approximation the frequency of nerve discharge at any instant may be taken as a measure of the intensity of the sense organ's excitation obtaining at that moment, or vice versa.

For the present purpose, and without experimental evidence on the point, sense organ excitation may be assumed to be inherently non-rhythmic and not all-or-none.\* The time-intensity course of such excitation may be

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\* No one in fact knows how a sense organ responds, our picture of the process is derived by inference from the response of the fiber activated by it. The possible exception is the visual sense cell, the potential of which was recorded by Hartline (8) in the eye of *limulus*, separate from the potentials of nerve elements associated with the sense organs. If this directly recorded potential is a measure of the state of excitation of the receptor cell, in the same sense that a nerve impulse seems to be a measure of its fiber's response, then the nerve fiber from a photo-receptor responds repetitively to a persisting and non-periodic excitation in its sense organ.

The other view is presented by Matthews (12) in connection with a study of muscle stretch endings. He infers that the sense organ itself responds repetitively to constant stimulation, i.e., physical stretch distorting the excitable membrane, "since there is no

process further, to the effect that a first stage might consist of the production of an exciting agent by non-nervous tissue, the second stage, of the excitation of a specialized nerve ending, which then activated its axone. For purposes of analysis then the peripheral sensory process can be considered physiologically at least as a three-stage one: first, the transformation of energy from a special to a general form, which makes the sense organ specific for a certain type of stimulus; second, the utilization of that energy in the setting up of a persisting state of excitation; and, third, the transformation of a persistent excitation into a repetitive train of impulses. It would be convenient, even if not experimentally justifiable, to identify as the loci of these stages, the non-nervous tissue, the expanded sensory nerve ending, and the nerve fiber respectively. The nerve fiber at least is in character, for it customarily responds with a repetitive train of impulses to a persistent stimulus, whether this be a constant current, a local injury, or a chemical agent.

Certain features of the sensory response to electrical shocks indicate that it is not the nerve fiber itself which is directly stimulated. The long latency of threshold prick is probably a function of a slowly established peripheral process, not characteristic of nerve fibers activated by brief shocks, and the narrow precision with which certain prick "high" spots and all hair endings can be located argues for the activation of a discrete and limited structure, not a random segment of a nerve plexus such as terminal fibers are known to pass through. In plotting touch areas, one occasionally finds, with strong stimuli, that the area to which the sensation is referred is a distant one, that is, a small superficial nerve branch has been stimulated directly (Fig. 3). The fact that with weaker stimuli touch areas can be accurately circumscribed with the stimulating needle without reference of any sensation to an external area is convincing by contrast. To stimulate prick *fibers* in such superficial nerve branches requires a stimulus so exorbitant as not to be comparable to the shock required for prick *receptors* under the needle, which are in contrast the most easily stimulated of all skin endings. It must be concluded that specialized endings, especially for prick, are more easily stimulated by electric shocks than the fibers leading from them. If as inferred above, one impulse in one fiber fails to arouse any sensation at all, then the ability to detect sense organs by such brief shocks as we have used itself indicates that specialized endings rather than fibers are being stimulated, for these shocks at any reasonable intensity could only set up one impulse each in any fiber they reached.

Finally, the distribution of endings correlates fairly well with the terminal nerve ending distributions of the skin to which Woollard and collaborators (17, 18) assign the functions of touch and pain. However, the data presented herein are not sufficient to conclude whether the touch endings lie more superficially than prick endings. Their failure to find any specialized endings for the fibers which they assign to pain may compromise

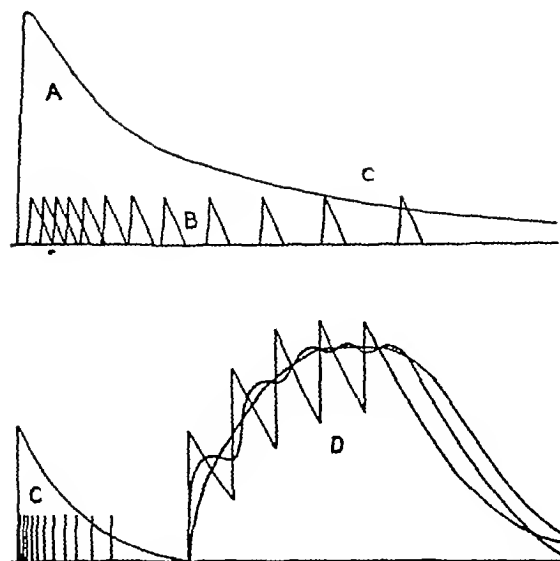


FIG 4

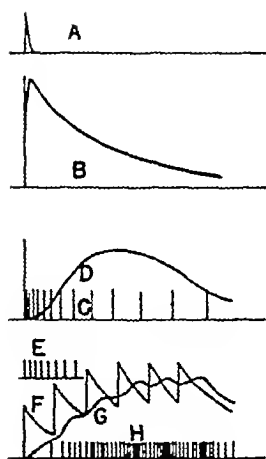


FIG 5

FIG 4 Diagram to illustrate the relation between sense ending and nerve, or between afferent fiber and the synapse to which it leads, on the assumption that the excitation in sense organ and at the synapse is persistent, in contrast to the discontinuous character of nerve fiber impulses. *A*, the triangles of constant amplitude represent a train of impulses in one fiber, decrementing in frequency. The overlying curve represents the course of the excitation in a sensory ending set up by a very brief stimulus and persisting at a decrementing intensity after the stimulus is removed. The decrementing intensity of the excitation of the sensory ending corresponds to the decrementing frequency of nerve impulses of constant intensity. It is supposed for purposes of representation that summation of the nerve impulses at a central synapse will reverse this transformation, resulting again in a persisting excited state of decrementing intensity.

*C*, the initial figure is a duplication of *A* on a slower time scale, the triangles represented as vertical lines. The saw-tooth curve represents a succession of such responses to a repetitive stimulation of the sensory ending, assuming that the frequency of stimulation is such that recovery is not complete between stimuli. The undulating and smooth curves superimposed on this represent increasing degrees of smoothing of the summation of impulses at a center, depending upon its character or upon the number of synapses traversed, etc. A repetitive excitation may thus be smoothed to give a continuous sensation, which may persist after stimulation ceases as the after-discharge of the summated excitation.

FIG 5 Diagrammatic representation of the pattern of excitation at successive levels of the nervous system, based on the assumption made in Fig 4. *A*, the potential-time form of a brief electric shock applied to a sense organ. *B*, the intensity-time course of the sense organ's excitation. *C*, the repetitive response of the afferent fiber to stimulation of its sense organ, assuming that the frequency of that response is at any moment proportional to the intensity of *B*. *D*, the smoothed curve of summation of *C* at a synapse, assuming that several impulses are required to excite it, involving a delayed rise of excitation. *E*, a train of impulses corresponding to *C*, condensed to the time of the first peak of the saw-tooth curve *F*, representing nerve fiber and sense organ excitation respectively. The whole curve *F* represents the summation of a succession of curves *B* upon repetitive excitation of a sense organ. *G* represents the summation of successive responses such as *D* at a synapse. *H* is then the series of impulses in the nerve fiber beyond the synapse, responding to the varying intensity of *G* with a periodically varying frequency. The delayed start of this train of impulses corresponds to a long latency of sensory effect, and the varying frequency of *H* corresponds to a vibratory character for the sensation. When the changes of frequency

then considered to have the form of the smoothed summation of the all-or-none responses of its nerve fiber, as indicated in Fig 4AB The effect at any synaptic region may again be evaluated as a smoothed summation of those same impulses, unless they are spaced too far apart to be summated smoothly, or at all \* How exactly the curve of intensity of subjective sensation will duplicate the curve of excitation of the sense organ, will depend on how the pattern of impulses in nerve fibers is modified as it passes central synapses, for instance, by the resistance of the synapse to single impulses

With a qualification to be noted below, the repetitive response of a nerve fiber following a single brief stimulus applied to a sense organ for touch or prick does not induce a repetitive *sensation*, that is a vibratory sensation This means that the lowest frequency at which the sense organ can cause the nerve fiber to repeat, is still too high for resolution by higher centers as repetitive sensation, and indicates an upper limit on the rate at which repetitive stimulation can be recognized as such Employing repetitive

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reason to suppose that the end-organ differs essentially from any other excitable structure," and he assigns to it a refractory phase similar to that of nerve, as a property of its polarisable membrane He obtained no records from the sense organ itself

\* This summation picture is suitable for the present representative purposes, whether summation at synapses takes place actually by building up a persisting excitatory state in the neurone, or whether it involves the activation of auxiliary pathways which reinforce a first impulse by delayed impulses, or whether both events occur in complicated centers Concerning the actual nature of a presumed central excitatory state, that is, a persisting state of excitation at a synapse or group of synapses, support for both of the above views may be found in recent literature For instance, Lorente de Nó (11) has shown that in the oculomotor system a volley of impulses may take two paths, one direct to a synapse causing an immediate post-synaptic volley, and one by a roundabout or "reverbering" circuit over which impulses arrive later at the same synapse, causing a scattered after-discharge On the other hand, Bronk (6) has shown that cells of the single-synapse station in the stellate ganglion are themselves capable of after-discharge following adequate repetitive stimulation, indicating a persistence of excitation at each synapse Whatever the actual process occurring at a synapse or at a group of them, its effect on post-synaptic fibers can be represented as a smoothed curve, and this curve may be interpreted according to the predilections of the reader as a persisting excitatory state, or as the mean of a series of discrete excitations

A reconciliation of these two views may be derived from some work of Lloyd (9a) on efferent nuclei activated via the pyramidal pathways of the cord Certain large cells whose axones could be discretely recorded from, responded only after considerable summation of impulses from adjacent units, and then responded with a rhythm different from that of the initial stimulus to the pyramids Lloyd remarks, "It is probable that the highly asynchronous activity of the small units in this region constitutes a slowly changing, statistically smooth over-all excitation to the large solitary cell units, and that these latter in turn respond at intervals determined in part by their own properties" This leaves undefined the exact locus of initiation of the all-or-none type of response, which for the time being is just as well

The significant point for the present discussion is the fact that impulses meet with something like inertia or impedance at synapses, such that an increasing effect is produced by successive impulses, and the energy used in overcoming this inertia may be later delivered as an after-discharge In this the synapse is similar to a sense organ, for instance a prick ending where an instantaneous stimulus may cause an effect only after a latency, but the effect persists long after the stimulus is removed The general result is a damping of the excitation sequence as in Fig 4, B D-G



start, in contrast with the slow rise of the sensation of prick. The adaptation to steady electrical stimulation is definitely less for either touch or prick than is that to steady mechanical pressure, which suggests that the chief adaptation is in the initial sense organ process, while electric stimulation may act beyond this, on a later stage of the excitatory sequence.

More pronounced changes in quality are correlated with changes of frequency and intensity of a repetitive stimulus. Well above threshold, a single shock to either a touch or a hair ending induces a brief but definitely rough feeling, as if it consisted of a short period of rapidly repetitive stimulation. This is not duplicated by mechanical stimulation. With high-frequency shocks this roughness disappears. It seems possible that this sensation reflects a real repetitive discharge in the nerve fiber, each impulse in the brief train which follows a single shock registering discretely in the central nervous system, until the rate of stimulation exceeds that which the centers can follow. Failure of this phenomenon with mechanical stimulation could then be accounted for by the circumstance that skin deformation would usually stimulate more than one ending, even if only of one axone, and the impulses reaching the centers would not be so regularly timed. No such impression is ever induced by any stimulation of prick endings.

The most striking change of quality is derived from the latter. A single stimulus little above threshold induces only a slight prick, if strengthened, a sharper prick is followed by definite itch. If repeated, at 5 or less per sec., the prick seems to be constant but the after-effect of itch builds up with successive stimuli. Various combinations of frequency and strength were therefore tried, with the aim of inducing only a feeling of itch from a single ending, without any initial pricking pain. This result is readily obtained with the combination of weak shocks and high frequency, shocks so weak that a single one is not painful, and several are required to summate for a definite itch, and frequencies anywhere from 10 per sec. up to a steady arc discharge. The higher the frequency, the less the strength must be for the purest sensation of itch, except that a steady current seems to be less effective than a fairly high frequency. After a few seconds of effective stimulation the itch persists for many seconds, and just below intensity required for frank pain, it is slight exaggeration to describe it as intolerable. Stronger stimulation causing pain is less disagreeable, and the itch is promptly allayed by scratching.

Attempt to induce tickle were less successful. Weak and rapid stimulation of hair endings came nearest to it, touch endings failed completely. In a few instances where a prick spot near a hair shaft happened to give the right combination, the tickle seemed to be enhanced by what would otherwise have been a slight itch. This together with common experience might lead one to infer that tickle requires stimulation of more than one ending, perhaps of more than one kind of ending, and could be compounded of touch and itch in the right proportions,—and especially if experienced against a more re-

direct nerve stimuli to skin nerves in human subjects, touch fuses into a smooth non-repetitive sensation at not over 60 per second (9) Pain fuses at 30 per sec, even with strong nerve stimulation With repetitive stimulation of prick *endings*, each individual stimulus of which obviously sets up a train of impulses as compared to the single impulse of direct nerve stimulation, so that summation readily takes place, fusion of the sensation occurs at a lower frequency than with stimulation of the nerve itself The condition can be diagrammed as in Fig 5 H, where repetition as a sensation is a function of the periodic fluctuation in frequency of nerve fiber discharge This is analogous to the conversion in radio receivers of a frequency-modulated carrier wave to an amplitude-modulated audio wave This is essentially the picture obtained by Bartley (4), for visual flicker fusion as observed in electrical records from the optic cortex

In fact, by pushing this analogy a little farther than experimental evidence at present warrants, the passage of a sensory message from the periphery to the center may be pictured as an alternation of amplitude modulation of excitability in cells or junctions, and frequency modulation in nerve fibers connecting them The character of the neural message is then expressed in terms of frequency modulation of all-or-none, that is maximal-amplitude, nerve impulses in the fiber pathways, and this will be converted to amplitude or intensity modulation at each cell station

This scheme, whether literally correct or not, may be employed as a convenient graphic representation of the results of experimental procedures It suggests the application of patterns of stimuli to single sense organs or definable groups of them, to test how the central nervous system deals with such patterns, as revealed in the pattern of sensation resulting The apparatus at present available delivers only the simplest patterns, regularly repetitive stimuli of controllable number, frequency and intensity, adequate for activation of one ending at a time Its limitations can be obviated at the expense of only a slight mechanical complexity, but it is suitable in present form for studying some of the attributes of sensory response

#### RELATION OF QUALITY OF SENSATION TO PATTERN OF SENSE ORGAN STIMULATION

At any given strength or frequency, the variation in number of stimuli, that is in duration of stimulation period, has little or no effect on the quality of sensation after the first few shocks The sensation simply persists, with minor changes in intensity of an adaptive character Prick builds up to a maximum and then decreases slightly Touch feels like an initial tap, with continuing steady pressure if the frequency is high Hair touch stimulation produces a constant sensation with little or no decreases after the abrupt

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of H become too rapid, or too slight to be followed by higher centers, the sensation will be a continuous one, that is the summation will have been completely smoothed as indicated in Fig 4 D

On the other hand, the interpretation put upon investigations of sensory response has been largely colored by subjective reports of localization of the stimulus, and by the naive inference that the finding of a sensory "spot" that could be identified uniquely, indicated that this spot might be innervated by a single fiber, connected uniquely to a corresponding spot in the cortex. This idea has broken down completely as applied to the one sensory area where acuity of spatial discrimination is most refined, that is in the retina. Not only is the overlap of innervation in the neurones of the layers of the retina itself enough to preclude it, (8) but further overlap takes place at the geniculate level (11a).

The work cited on innervation of the skin suggests that here also the subjective recognition of discrete loci of stimulation is not to be accounted for by simple one-to-one connections between sense organs and cortical neurones. While points of extreme sensitivity are found in the skin, for both touch and prick, by electrical stimulation as well as mechanical, it does not seem probable that these points are the *unique* loci of the characteristic end organs capable of giving the same sign. Around each prick spot, however sharply localized, prick can be induced by a slight increase in current strength, and around each touch spot is a touch area capable of similar activation. The differences in strength of shock over positions having different thresholds are not sufficient to account for stimulation of one central spot by diffusion of current, the unit such as it is, is an area covered practically continuously by sensory endings, and apparently by endings of more than one fiber.

The apparent uniqueness of sensory foci must then be a function of the relative thresholds of such endings, due either to differences of intrinsic sensitivity or to differences of accessibility to the stimulus, etc. Woollard *et al* (18) emphasize in fact that even with mechanical stimuli, localization must be considered to be three-dimensional. The notorious difficulty in identifying histologically the unique endings in excised areas in which sensory spots had been previously located may be accounted for in part by the multiplicity of such endings, the apparent uniqueness being a function of accessibility to stimulation.

To the extent that such considerations are valid, the distinctions that are tacitly made between mechanisms for general skin sense and for special sense may be dispensed with, and with them may be abandoned the notion that any modality is primitive, in the sense that it is served by a simpler or less elaborate neural mechanism. Rather one may look for a general scheme in accordance with which the nervous system receives and handles afferent messages, and differentiates between the intensity, quality, and spatial extent of an excitation. The function of hearing a sound of frequency above what any one nerve fiber can transmit raises the same problem as that of recognising a mechanical vibration of frequency higher than any one skin touch organ seems able to follow, and the explanation of visual acuity, when arrived at, should apply to the problem of skin localization. The findings of

ceptive emotional background than that which motivates its dispassionate analysis

An occasional hair ending which could be stimulated to give hair-touch alone, without either pressure or prick endings being activated, showed a peculiar partial adaptation, in that, when stimulated slightly above its threshold at high frequency, the sensation changed in quality during the course of the constant-strength stimulation. The initial effect was identical with that caused by bending a hair, but this sensation faded into a mild tickle, which persisted as long as the stimulation, without significant after-effect. This is in marked contrast to similar stimulation of prick spots, which caused an increasing itch with a long-persisting after-effect. To describe the one as due to partial adaptation, the other as due to summation, implies that these two processes are simple opposites, and this may be an over-simplification, for the hair-endings seem to show adaptation in the sense of responding more weakly after an initial period, but also summation in the sense that a repetitive stimulus results in a non-repetitive sensation. Pending actual observations of nerve impulses in animals after similar stimulations, these terms should be taken only in a descriptive sense.

### DISCUSSION

An adequate understanding of the physiological basis of sensation would require a knowledge of the activity as well as of the structure of each unit along a path between the sense organ and the cortex, to match against a report of the sensation involved. Most of the descriptions of the activity of parts of the central nervous system have been derived from synchronous stimulation of many parallel fiber pathways, on the presumption that the responses of similar elements synchronously activated would be equivalent to the activity of one element of pathway stimulated through a single fiber. This is certainly only a first approximation to the truth, even allowing that one *element of pathway* through the central nervous system probably involves a number of parallel neurones. Recent anatomical work indicates that even in the sensory periphery the functional unit of pathway is not a single fiber and its end-arborisations. Weddell (15, 16) reports that groups of Meissner's corpuscles not over 0.15 mm. apart and innervated each by a separate fiber, constitute a touch spot, and that each hair may be innervated by two separate fibers. Further the areas innervated by the end terminals of fibers presumably mediating pain in the skin overlap one with another. In the cornea Tower (13) found that stimulation of any area within the field of one fiber caused that fiber to respond, and one fiber and its branches extended over as much as one quarter to one half the cat's cornea, plus neighboring regions of the sclera, as indicated by recorded responses of single fibers to mechanical stimulation of the cornea. Here again overlap occurred between fiber fields. The pathway for sensation is apparently multiple even from the periphery.



Derbyshire and Davis (7) that the auditory nerve fibers respond alternately when the frequency of stimulation exceeds the capacity of any one fiber, and of Bartley (2) that the cortex responds at half frequency when the frequency of light flashes to the retina exceeds the ability of the cortex to respond to each, indicate one expedient employed by the nervous system to overcome the limitations of its individual elements with respect to frequency of response. The scheme hypothesized by Lorente de Nó (10) designated as "partially shifted overlapping" proposes another by which it might deal with the limitations of spatial distribution. It may be anticipated that recognition of quality of sensation, exemplified crudely in peripheral terms as difference in local sign, will involve more than a spatial localization in the brain of projection areas corresponding to sense modalities, although the neural mechanisms actually appropriate to this function have not yet been suggested. The notion that pain is a function of the thalamic centers and discrimination that of the cortex is presumably only the same sort of too simple rationalization to account for quality of sensation, as is the assumption of point-to-point correspondence between brain and periphery for spatial discrimination.

Even though electrical stimulation of one ending of one fiber may be possible, it does not follow that the central nervous system transmits and interprets the impulses induced in a correspondingly simple manner. The chief virtue of such experimental restriction of activity to one peripheral element may be the demonstration that such stimulation is insufficient to enable the central nervous system to obtain an adequate estimate of external conditions. It may be inferred that the central nervous system is designed not primarily to detect discretely the activity of each ending, however precisely it may incidentally accomplish this, but rather to integrate the activities of many, and even peripheral sense organ activity will not be satisfactorily analysed, at least in terms of subjective sensory response, without a further understanding of the central mechanisms that react to it, as well as a knowledge of what each sense organ does to its immediate nerve fiber.

#### SUMMARY

Single sensory spots in the skin of human subjects can be conveniently stimulated by high voltage, low current spark discharges, without mechanical deformation of the skin.

The distributions of sensitivity over various regions, for touch and prick, show characteristic patterns. "High" spots of extreme sensitivity to electric stimulation are surrounded by areas of lower sensitivity. An area, varying in size in different regions, from less than two mm. to more than fifteen mm., appears as a unit in the sense that any stimulus within it is referred to the same locus.

Prick has a much lower threshold than touch, except on the balls of the fingers, where touch threshold is lower.

Tactile endings associated with hair shafts can be differentiated from

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limit is imposed by the circumstance that a certain initial quantity of current is utilized in ionizing the air in the gap, after which the arc resistance is abruptly lowered. A condenser small enough to suit the present purposes loses so large a fraction of its current, even at high initial voltage, in ionizing the gap that the residual voltage after ionization is not sufficient to cause an arc, or if the initial quantity of current is increased, the subsequent arc discharge through the lowered resistance may be above threshold for a sense ending. If there is a critical value that would be theoretically suitable, its range is too narrow to be practical. A value of capacity and voltage is therefore employed which is safely above this critical and unstable level, and part of the current may be wasted in the shunt circuit, which acts as a fine adjustment.

To overcome the inconstancy of operation of this spark, the constant current delivered through the pentode and switch *H* is held just at discharge level, indicated by a faint buzz in the phone, and this current is far below threshold for skin sense organs. The variable condenser *F* is also charged by this steady current in one direction, through the grounded resistance to its left hand terminal, it is discharged and charged in the opposite direction by the impulse, adding the two voltages at the moment of discharge, and permitting the use of a very small condenser with a short time constant in the transient branch of the circuit.

Two thousand ohms are shunted across each ear phone, to permit operation if the plug is not inserted. The tap key *N* employed to signal latencies of sensation, operates by grounding the oscillograph input, the amplitude of this signal depending on the value of resistance in series with *N*. The short-circuit switch *K* is on a rotating interruptor which also controls the oscillograph sweep, and by adjusting the duration of the opening of *K*, one or more of a sequence of repetitive shocks are applied to the skin and recorded on the oscillograph at each revolution of the interruptor. The apparatus is constructed to deliver currents making the stimulating needle at *Z* cathodal, but the anode seems to be equally effective and construction might be simplified by reversing the polarity of the stimulus generator. The body has sufficient capacity so that a ground connection is not necessary, except to carry the impulses to the recording apparatus. The spark gap works more constantly if the anode is needle-pointed. The condenser at *F*, of the radio transmitting type, has a maximum capacity of about 0.0001 Mf, and fixed condensers can be inserted in parallel for stronger stimulation. The design and construction of the apparatus are due to Mr. R. G. Loeffel.

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was tested by Gerard and his associates (9, 10) In ordinary nerves, however, only general estimates are possible since both electrical and chemical changes are too small for such studies The large electric organ of *Electrophorus electricus* offers a suitable material for comparing electrical and chemical changes of the action potential since both are in the range of possible measurement Experiments carried out on these organs and described in this paper show that the energy mechanism of the action potential is fundamentally identical with that used in muscular contraction

#### METHODS

a *Phosphates* The method of Fiske and Subbarow as applied by Lohmann (16) was used The tissue frozen in liquid nitrogen was ground in iced trichloroacetic acid (5 per cent) and filtered For the determination of the true inorganic phosphate an aliquot portion of the filtrate was taken and within less than three minutes added to a solution of ammonium magnesium citrate prepared according to Mathison (17) Another fraction of the filtrate was directly analyzed, indicating the true inorganic phosphate and phosphocreatine phosphate A third fraction was hydrolyzed in *N* HCl at 100°C for 15 min to obtain the phosphate of adenosinetriphosphate For the determination of the total phosphate one fraction was ashed with 2 *N* sulfuric and nitric acids A photoelectric colorimeter was used

b *Lactic acid* The method described by Barker and Summerson (2) was used in which a pink color was developed by p-hydroxydiphenyl This determination also requires a photoelectric colorimeter

c *Creatine* The creatine was transformed into creatinine and determined by Fohn's open flask method

#### RESULTS

A *Distribution of phosphorylated substances and creatine* The presence of phosphocreatine in the electric organ was first described by Kisch (13), who suspected that this compound might be connected with the activity of the organ Baldwin and Needham studied a series of enzymatic reactions in extracts of the electric organ, particularly those connected with phosphorylated compounds They showed that some of the main steps known to occur in intermediate muscle metabolism also occur in those extracts (1)

The observations mentioned above were carried out on the electric organ of *Torpedo* In the electric organ of *Electrophorus electricus*, the concentration of phosphocreatine is as high as in striated muscle and in some cases even considerably higher (see Table 1) In specimen No. 1 the values of phosphocreatine are on the average about 2 mg  $P_2O_5$  per gm, which is a concentration about 50 per cent higher than in frog's muscle This is particularly remarkable in an organ with 92 per cent water content The concentration of adenosinetriphosphate (ATP), although lower than in muscle, is still high 0.2–0.4 mg  $P_2O_5$  of ATP phosphate In contrast to the variations of choline esterase the distribution of phosphate and phosphorylated substances does not decrease from the head to the caudal end of the main organ

Creatine is also evenly distributed and the concentration is higher than in muscle and in the electric organ of *Torpedo* About 500–600 mg per 100 gm of electric organ were found in medium sized specimens as compared with 300–400 mg in striated muscle In the Bundles of Sachs, which releases much less energy per gm, than the main organ, both phosphocreatine and creatine

# ACTION POTENTIAL AND ENZYME ACTIVITY IN THE ELECTRIC ORGAN OF *ELECTRO- PHORUS ELECTRICUS*. II PHOSPHO- CREATINE AS ENERGY SOURCE OF THE ACTION POTENTIAL

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## INTRODUCTION

RECENT investigations suggest that the release of acetylcholine is intrinsically connected with the nerve action potential and may be responsible for the electrical changes occurring at the neuronal surface during nerve activity (7, 19-24). The new concept of the physiological role of acetylcholine is based mainly on the following facts: (i) on the high concentration of choline esterase in nerves, which shows that significant amounts of acetylcholine can be metabolized within milliseconds, (ii) on the localization of choline esterase at the neuronal surface, and (iii) on the close parallelism between the concentration of choline esterase and the maximum voltage of the action potential which may be demonstrated on electric organs.

The changes at the neuronal surface during activity and their rapid reversal cannot conceivably be effected without energy loss, since they must involve processes which are—from the thermodynamic point of view—irreversible, and can only be reversed by the free energy of chemical reactions. If the release of acetylcholine and its subsequent breakdown is responsible for the alterations of the nerve membrane during the transmission of the nerve impulse, chemical reactions must supply the energy for the resynthesis of acetylcholine.

The most readily available source of energy for endergonic life processes is the energy of phosphate bonds. In muscle, the breakdown of adenosinetriphosphoric acid appears to be the primary energy source for the contraction, possibly directly connected with the mechanical changes (4, 18). The adenosinediphosphate formed is rephosphorylated by the breakdown of phosphocreatine, a phosphate shift which occurs without loss of energy. Phosphocreatine thus acts as a "storehouse" or "accumulator" for energy rich phosphate bonds (14). Ever since phosphocreatine was found in nerve it was assumed that it may have a function there similar to its function in muscle and may yield the energy for the action potential (6, 8, 11). This possibility

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The number of discharges during each period of stimulation was roughly counted by watching a cathode ray oscillograph connected to the electrodes

Three such observations are recorded in Table 3. The experiments show that the developments of the action potential are associated with breakdown of phosphocreatine. Moreover, at the time when the fish shows marked fatigue, phosphocreatine has fallen to a low level. In Expt no 3 for instance the organ appeared during the second period of stimulation to be very fatigued. The phosphocreatine level was already low at that period. When, after 3 min rest, stimulation started again, the organ appeared nearly completely fatigued and during twelve minutes of prodding only a few discharges of low voltage occurred. During this period the phosphocreatine did not change measurably. 0.39 and 0.35 mg  $P_2O_5$  of phosphocreatine phosphate respectively were found.

The rate of recovery appears to be slow. In experiment 1 for instance there is no measurable resynthesis of phosphocreatine after 5 min rest on the table and 20 min recovery in water. Only after 20 more min in water a synthesis had occurred. The initial value was in this case rather low, so that the synthesis is still rather small, although the amount is nearly as high as before the stimulation period.

*C Correlation between electric energy and chemical energy released by breakdown of phosphocreatine.* By the experiments just described the breakdown of phosphocreatine was linked to the discharge. A quantitative comparison was next undertaken between the energy released by the breakdown of phosphocreatine and the electric energy delivered to the external circuit in a large number of discharges. The number of discharges was counted by means of an electric "scaling circuit" by which every sixteenth discharge was indicated. The oscillograph was used with a camera to obtain photographic records of the voltage. A number of exposures were made at different times during the period of stimulation. The electric power released externally is  $V^2/R$ , where  $R$  is the resistance of the external circuit and  $V$  is the voltage between its terminals. The voltage varies continuously during the impulse but is shown at each instant by the oscillographic trace. The instantaneous power could thus be computed at a number of different instants during the discharge, and from this computation a graph of the power vs the time could be drawn. Since the energy is the time integral of the power, its value could be found by a graphical integration of the power-time graph.

The cross section of the organs could not be measured directly, since the fish were not killed, but the girth of each fish was measured and the cross-sectional area was estimated by comparison with sections of other electric eels on the assumption that the area is proportional to the square of the girth. The fish were all of similar size, so that the relative areas were probably determined in this way with fair precision. From the average energy of one impulse and the estimated volume, the energy per impulse per gm of electric tissue was computed, the specific gravity being taken as unity.

are markedly lower than in the main organ. Only 0.3–0.4 mg.  $P_2O_5$  were found as phosphocreatine per gm of electric organ. The creatine content was 250–300 mg per 100 gm. No ATP was found, although it is probable that the compound is present and that the amounts are too small for determination by indirect estimation in the presence of the other phosphates.

Table 1. Concentration and distribution of phosphocreatine (PhCr) and adenosinetriphosphate (ATP) in the electric organ of *Electrophorus electricus*. Four samples of the second specimen were taken from the Bundles of Sachs (B o S).

Specimen No	Distance from the anterior end cm	$P_2O_5$ directly determ mg /gm	PhCr in mg $P_2O_5$ per gm	ATP in mg $P_2O_5$ per gm	Total $P_2O_5$ mg /gm
1	5	3.17 2.98	2.23 2.08	0.19 0.20	3.43 3.03
	8	3.15	1.76	0.19	3.60
	25	3.30 3.07	2.41 2.14	0.16 1.10	3.57 3.57
	30	2.94	1.90	0.25	3.32
2	6	2.61 2.40	— 1.40	0.39 —	3.16 3.11
	8	2.35 2.72	1.39 1.27	0.32 0.39	— —
	27	2.35	1.50	—	2.76
	30	2.54 2.54	1.27 1.21	0.39 0.31	— —
	58 B o S	0.59 0.59	0.43 0.37	0 0	0.74 0.84
	63 B o S	0.57 0.54	0.34 0.37	— 0	— —

**B Breakdown of phosphocreatine as result of discharge** A few preliminary experiments were carried out to see whether phosphocreatine is split as a result of the electric discharge. The fish was taken out of water and laid in an insulating trough in which two electrodes 10 cm apart made contact, each over an area of some  $cm^2$ , with the skin covering the main electric organs. These were joined by a resistance of 30 to 100 ohms in order that the discharge should release energy by an electric current. A piece of electric organ from the part between the electrodes was rapidly cut out and thrown into liquid nitrogen. After varying periods of stimulation by light prodding alternating with periods of rest, other pieces were cut out and similarly frozen.

In the first experiment, the number of discharges was 320. The resulting breakdown of phosphocreatine was small for quantitative measurement, and in the next two experiments the number was increased to 800. The energy of the phosphocreatine breakdown per gm and impulse in these three experi-

Table 4 Breakdown of phosphocreatine (PhCr) in the electric organ of *Electrophorus electricus* during the discharge. Number of discharges 1600 a before, b after period of stimulation c of exp No 6 was taken after 65 min recovery in water. Figures separated by a line indicate values obtained by using different pieces, otherwise control determinations by using extracts of the same piece

Exp No		1		2		3		4		5		6			7	
		a	b	a	b	a	b	a	b	a	b	a	b	c	a	b
P <sub>2</sub> O <sub>5</sub> directly determined mg /gm		2 00 1 03	1 88 1 01 1 83	1 64 1 93	1 77 1 76	1 82 1 78	1 80 1 81	1 92 1 95	1 09 1 80	2 23 2 24	2 13 2 25 2 12	2 24 2 07	2 08 2 07	2 11 2 12	1 06 1 05	1 00 1 08 1 08
PhCr in mg P <sub>2</sub> O <sub>5</sub> per gm	single	1 10 1 14 0 98 1 01 0 57	0 55 0 41 0 46 0 32 0 57	0 84 0 84 1 12	0 52 0 47 0 57	0 88 0 80 0 82	0 64 0 68 0 83	1 28 1 28 1 33	1 02 1 01 0 96	1 60 1 60 1 60 1 60 1 65 1 66	1 18 1 17 1 18 1 08 1 11	1 42 1 41 1 34 1 33	0 75 0 75 0 67 0 62	1 03 1 01 1 10 1 10	0 55 0 70 0 56 0 65	0 10 0 10 0 42 0 40 0 43 0 47
	average	1 07	0 50	0 98	0 515	0 85	0 755	1 28	0 90	1 67	1 16	1 375	0 70	1 06	0 64	0 345
	splt	0 57		0 465		0 095		0 32		0 51		0 675			0 295	

Table 5 Breakdown of phosphocreatine (PhCr) in the electric organ of *Electrophorus electricus* during the discharge. Number of discharges 1600 a before, b after discharges

Expt No	Time of excision	P <sub>2</sub> O <sub>5</sub> directly determ mg /gm	PhCr in mg P <sub>2</sub> O <sub>5</sub>		Expt No	Time of excision	P <sub>2</sub> O <sub>5</sub> directly determ mg /gm	PhCr in mg P <sub>2</sub> O <sub>5</sub>	
			per gm	splt				per gm	splt
8	a	2 38	1 52	0 47	12	a	1 98	1 36	0 42
	b	1 89	1 05			b	1 92	0 94	
9	a	1 85	1 15	0 18	13	a	2 00	1 345	0 225
	b	2 10	0 97			b	2 27	1 12	
10	a	1 89	1 235	0 35	14	a	2 01	1 30	0 375
	b	1 99	0 885			b	2 05	0 925	
11	a	1 76	1 07	0 28	15	a	1 59	0 97	0 30
	b	1 60	0 79			b	1 65	0 67	

ments had the values 34.4, 36.9, 85.0 g Cal  $\times 10^{-6}$  as compared with values 9.9, 14.3, 7.0 for the external electric energy. Even with 800 discharges the breakdown of phosphocreatine was smaller than was desirable. Since no fatigue was evident during 800 discharges the number was increased to 1600 in all the following experiments. This number seemed about optimum, as fatigue, when it was detectable at all, was evident only toward the end of the

It was found that the electric energy was regularly near its maximum when the resistance was 100 ohms. Consequently this resistance was used in all experiments after the first few. In every experiment, immediately before and after the counted series of discharges, a piece of electric tissue was quickly cut out and thrown into liquid nitrogen and the phosphocreatine was later determined. The energy released per gm of electric tissue by the break-

Table 2 Concentration of creatine in different sections of the electric organ of *Electrophorus electricus*

Specimen No	1				2				3
Distance from the anterior end in cm	8	10	30	45	10	40	60	B o S	near ant end
mg tissue used	850	548	522	512	567	621	490	521	543
Creatine mg per gm	490	548	526	649	530	490	550	300	260
									669
									603

Table 3 Breakdown of phosphocreatine (PhCr) during the discharge of the electric organ of *Electrophorus electricus*

I a before, b after stimul (13 min, about 2000 discharges), c 5 min later (fish kept in air) d after 20 min in water, e after 20 more min in water II a before, b after stimul (5 min, about 500 disch) c 11 min rest, then 10 min stim (about 800 disch) d 7 min rest then about 200 disch, marked fatigue III a before, b after stimul (12 min, about 2000 disch) c 3 min rest, then 4 min stimul. (about 700 disch), marked fatigue, d 3 min rest, then 12 min stimul, nearly completely fatigued

Exp No		P.O <sub>4</sub> directly determ mg/gm	PhCr in mg P.O <sub>4</sub> per gm	ATP in mg P.O <sub>4</sub> per gm	Total P.O <sub>4</sub> mg/gm
I	a	1 89	0 81	0 23	2 15
	b	1 93	0 35	0 25	—
	b	2 04	0 49	0 17	—
	c	2 01	0 32	—	2 19
	d	1 94	0 27	0 25	—
	e	2 04	0 85	0 17	2 23
II	a	2 95	2 07	0 12	3 23
	b	3 00	1 57	0 22	3 26
	c	2 72	0 86	0 13	2 86
	d	2 85	0 96	0 19	3 10
III	a	2 23	1 23	0 12	2 35
	b	2 03	0 49	0 10	2 15
	c	2 06	0 39	0 10	2 16
	d	1 96	0 35	0 06	2 07

down of phosphocreatine was divided by the number of discharges to give the energy per gm per impulse for comparison with the electric energy reckoned on the same basis

control determinations were made of the true inorganic phosphate. Each value obtained was thus controlled several times. Since the two parts of the same piece generally showed a fairly good agreement, in the later experiments (No 8-15, Table 5) the pieces were not divided, but still two controls were always made from each piece. The figures given in Table 5 are mean

Table 7 Lactic acid (L A) formation in the electric organ compared with the breakdown of phosphocreatine and the electrical energy released externally by the action potential. Number of discharges 1600. External resistance 100  $\Omega$ . a) before b) immediately after stimulation c) during recovery

Expt No	mg tissue used	L A mg /gm	L A formed mg /gm	Energy released per gm & impulse Cal $\times 10^{-4}$			
				by L A formation	by breakdown of PhCr	by the two chemical reactions	external electrical energy
8	a 296	0 169	0 057	10 1	41 4	51 5	7 1
	b 305	0 226					
	c 600	0 221					
9	a 307	0 143	0 337	37 5	16 0	53 5	10 5
	b 305	0 480					
	c 323	0 489					
10	a 478	0 273	0 117	12 8	31 0	43 8	7 8
	b 447	0 390					
11	a 684	0 310	0 136	15 1	24 7	39 8	6 7
	b 424	0 446					
	c 890	0 445					
13	a 472	0 379	0 137	15 2	19 8	35 0	4 4
	b 339	0 516					
14	a 523	0 262	0 130	14 4	33 0	47 4	5 5
	b 718	0 392					
15	a 694	0 362	0 112	12 5	26 4	38 9	5 4
	b 514	0 474					
Average			0 147	16 8	27 5	44 3	6 8

values since the differences between the two controls were without exception not significant.

The piece taken after stimulation was at first excised from the same region as that for the initial value. Since it appeared likely that the hole made by the first incision would change the current in the adjacent region, in later experiments two completely separated cuts were made. However, no difference became apparent between these two procedures. The time during which the discharges occurred was relatively short. In the first few experiments it was generally 3 to 5 min, later it did not exceed 2 min. Since the

series and was even then usually slight. The amount of phosphocreatine split, on the other hand, was sufficiently large, yet the phosphocreatine did not fall to too low a concentration.

It cannot be expected that all specimens will be in the same condition. There is a short period—usually about one min—after they are removed from the water and before the first piece of tissue is cut. During that period

Table 6 External energy released by a section of the electric organ of *Electrophorus electricus* compared with the energy released by phosphocreatine breakdown in the same section during the same period. Length of section 10 cm. The external resistance was 100  $\Omega$ . Number of discharges 1600.

Expt No	Length cm	Measured section		Electrical energy externally released		PhCr split in whole section in mg $P_2O_5$	P energy in whole section Cals	PhCr split in mg $P_2O_5$ per gm	P energy per gm & impulse Cal $\times 10^{-4}$
		Girth cm	estim volume cc	in whole section Cals	Per gm & impulse Cal $\times 10^{-4}$				
1	185	36.6	400	9.2	14.5	228.0	32.0	0.57	50.2
2	179	36.0	380	5.9	9.7	176.5	24.9	0.465	41.0
3	182	36.5	390	5.6	9.0	37.8	5.3	0.097	8.5
4	185	39.0	440	5.6	7.9	141.0	19.9	0.32	28.3
5	161	34.5	350	5.3	9.6	178.5	25.1	0.51	44.8
6	167	38.5	430	5.4	7.8	290.0	41.0	0.67	59.0
7	172	35.5	370	5.0	8.5	109.0	15.4	0.295	25.9
8	171	37.5	410	4.7	7.1	192.5	27.0	0.47	41.4
9	182	36.7	500	6.7	10.5	72.0	10.2	0.18	15.9
10	183	35	360	4.5	7.8	126.0	17.8	0.35	31.0
11	182.5	38	420	4.5	6.7	118.0	15.7	0.28	24.7
12	171	37.5	410	5.3	8.5	172.0	24.2	0.42	36.3
13	161	37.5	410	2.9	4.4	92.0	13.5	0.225	19.8
14	190	38	420	3.7	5.5	157.5	22.2	0.375	33.0
15	181.5	36.5	390	3.4	5.4	117.0	16.4	0.30	26.4
Average				5.5	8.2	147.2	20.7	0.369	32.4

some of them may give many discharges, others only a few. Variations of the initial concentration of phosphocreatine have therefore to be expected independently of the individual variations which may occur in the initial value in optimal conditions.

The results of phosphocreatine determination of 15 experiments carried out with 1600 impulses are summarized in Tables 4 and 5. The variations are in reasonable limits and the deviations from the average value not greater than those usually found for the breakdown of phosphocreatine for a given number of twitches of an excised frog's muscle. Only in Expt no 3 an exceptionally low value was obtained which may be due, partly at least, to the relatively low initial value. In the first seven experiments (Table 4) each piece was divided in 2 or even 3 parts while frozen and with each part two



the total electric energy to that released by the breakdown of phosphocreatine

D *Lactic acid formation* Heat production during nerve activity was demonstrated at a period—in 1926—when lactic acid formation was still considered to be an essential and primary process of muscle contraction. The first investigations on a possible formation of lactic acid connected with nerve activity were either negative or doubtful. Holmes, Gerard and Solomon (12) found a slight rise of lactic acid in frog's sciatic after prolonged stimulation (5 mg per cent) but none in rabbit's nerve. Referred to the energy required the amount of 5 mg per cent, if correct, is quite considerable. But with the chemical methods then used it is close to the limit of possible measurement. From experiments on nerves poisoned with iodoacetic acid, Feng concluded, in 1932, that formation of lactic acid, although not essential to nerve activity, is required if the nerve is to endure prolonged activity in oxygen (5). Addition of sodium lactate to poisoned nerve improved its capacity for prolonged activity in oxygen. The facts were confirmed by Chang and Gerard (3).

The formation of lactic acid as a result of the action potential was studied on the electric organ. In the experiments No. 8-15 described in the preceding paragraph the pieces of electric tissue while frozen were split and one part was used for the determination of phosphocreatine, the other for lactic acid. After 1600 discharges the amount of lactic acid formed per gm of tissue was about 0.15 mg on the average (see Table 7). The resting value varied between 0.15 and 0.35 mg lactic acid per gr. If we assume that 1 mole of lactic acid formed releases 16,000 g Cals without neutralization heat, the energy supplied by the lactic acid formed is on the average  $16 \times 10^{-6}$  g Cals per gm and impulse. In the 7 experiments summarized in Table 7 the external electrical energy released per gm and impulse was on the average  $6.8 \times 10^{-6}$  g Cals, the energy released by the breakdown of phosphocreatine  $27.5 \times 10^{-6}$  g Cals. Thus the external electric energy amounts to only 15 per cent of that of the two chemical reactions. The actual ratio of external electrical to chemical energy is still smaller since at least the oxidation energy has to be added.

Since lactic acid formation is certainly a recovery process and could therefore occur at a delayed period, in a few experiments a third piece of tissue was cut 5-6 min after the end of the stimulation period. The fish was continuously under control of the oscillograph and the piece was not taken if no more than a few discharges had occurred. Experiments No. 8, 9 and 11 show that no delayed lactic acid formation occurred during 5 to 6 min after the end of stimulation. They also show that, at rest, no measureable lactic acid formation occurs during such a period.

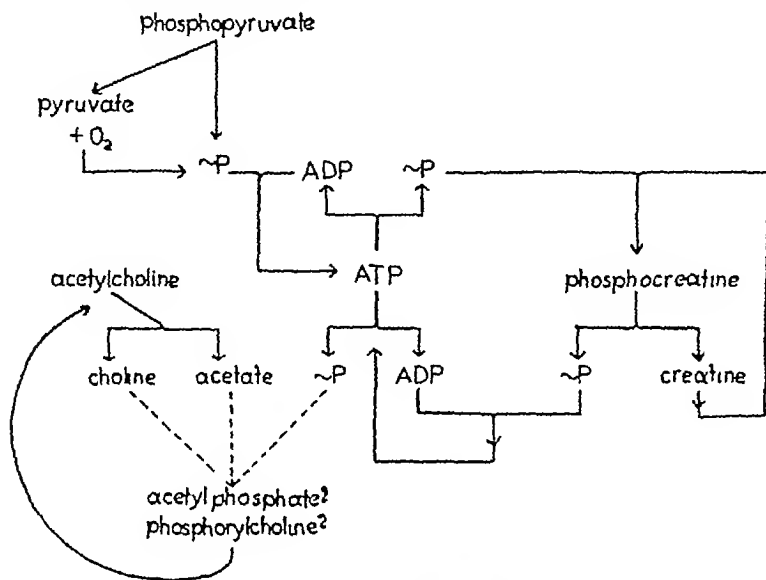
#### DISCUSSION

The experiments described above offer evidence that the breakdown of phosphocreatine is adequate to account for the electric energy released by

lactic acid formation is small and the rate of respiration low, the  $Q_{O_2}$  being about 0.3 to -0.4, it seems probable that during this period there is only a negligible resynthesis and that the amount of phosphocreatine actually split is not markedly different from that measured. This is supported by the result of Expt. no. 15 in which after 65 min rest in water only two thirds of the phosphocreatine split during the stimulation period was resynthesized. The observations on the external electric energy released by 1600 discharges are summarized in Table 7 and compared with the energy released by the breakdown of phosphocreatine. The calculation of the energy of the phosphate bonds is based on the assumption that one mole of phosphocreatine yields 10,000 g Cal.

Per gm and impulse the average energy released by the breakdown of phosphocreatine is  $32 \times 10^{-6}$  g Cal. The average external electric energy per gm and impulse is approximately one fourth of this, being  $8.2 \times 10^{-6}$  g Cal. It would be desirable to compare the energy released by the breakdown of phosphocreatine with the total electric energy produced by the organ instead of with the part only which was delivered to the external resistance. Any system which can be characterized by an electromotive force and an internal resistance will deliver maximum power to an external circuit having a resistance equal to the internal resistance. In this case the power spent in maintaining the current through the internal resistance is just equal to that delivered externally, so that the external power is half the total power. If the internal resistance is variable, as that of the electric organs probably is during the discharge, then it can not be matched at every instant by any constant external resistance. The maximum external energy will then be somewhat less than half the total energy. But measurements made on a number of oscillographic traces in the present experiments indicate that the ratio of the maximum external energy to the total will not be much different whether it is the internal resistance or the electromotive force or both which vary during the discharge. The reason for this is that the voltage rises rapidly to a maximum and then remains fairly uniform for most of the duration of the discharge, before falling rapidly at the end. Thus most of the electric energy is generated during a time when the electrical characteristics of the tissue are fairly constant, and consequently it makes little difference for the present purpose which characteristic is variable during the rest of the time. If therefore we could be sure that the external energy we measure is the only energy delivered outside the electric tissue, it would be possible to say that the total electric energy is about twice that measured externally and about half that released by the breakdown of phosphocreatine. But some energy must be spent in producing a current in the non-electric tissue, adjacent to the electric organs, and this escapes our present measurement. The conclusion from the observations made so far is that the maximum electric energy delivered to an external resistance is about one fourth, and the total electric energy at least half of that released by the phosphocreatine breakdown. Further experiments, however, are necessary to ascertain more definitely the ratio of

The adenosinediphosphate is immediately rebuilt to ATP by the breakdown of phosphocreatine. The creatine is rephosphorylated either by the phosphopyruvic acid, ATP acting again as intermediate ("Parnas reaction") or by the oxydative breakdown of pyruvic acid. Since the release of the ACh starts the whole chain it may be called the "acetylcholine cycle." It has still



Description of Figure

The "acetylcholine cycle"  
 ATP = adenosinetriphosphate  
 ADP = adenosinediphosphate  
 ~P = energy rich phosphate bond

to be ascertained which may be the intermediate phosphorylated link between ATP and acetylcholine. One possibility is the formation of acetylphosphate, the compound described by Lapmann (15). Phosphorylcholine may also be the intermediate exchanging its phosphate with acetate.

#### SUMMARY

Breakdown of phosphocreatine and formation of lactic acid as a result of the discharge were determined on the electric organ of *Electrophorus electricus*. The energy supplied by these two chemical processes was compared with the electrical energy released. The following results were obtained:

1. The external electrical energy per gm. of tissue and impulse is on the average  $8.2 \times 10^{-6}$  g. calories. The total electrical energy is at least twice as high. The amount of phosphocreatine split supplies per gm. and impulse on the average  $32.8 \times 10^{-6}$  g. calories, that is four times as much as the external electrical energy.

the action potential of the electric organ. The externally released electric energy per gm and impulse is about 8 microcalories, the total electric energy at least twice as much, whereas the phosphocreatine split yields about 32 microcalories. The heat production observed in ordinary nerves is of the same order of magnitude as the energy changes observed in the electric organ. The total heat production in the crab nerve per gm and impulse is about  $35 \times 10^{-6}$  g Cals. In frog's nerve it is about  $4-5 \times 10^{-6}$  g Cals at  $0^{\circ}\text{C}$  and  $10^{-6}$  g Cals at  $20^{\circ}\text{C}$ . Quantitative differences are always possible even if the process is fundamentally identical. The phosphocreatine content of nerve is markedly lower than that of the electric organ, although referred to the active surfaces the difference may be small. The heat production in electric tissue has not yet been measured. It will probably be somewhat higher than in crab's nerve since phosphocreatine breakdown and lactic acid formation already yield  $44 \times 10^{-6}$  g Cals per gm and impulse and the oxidation heat has still to be added. But the order of magnitude is still the same. Former observations suggest that the primary alterations during the action potential are connected with the release and breakdown of acetylcholine. The present observations are consistent with the conclusion that phosphate bonds may be used to resynthesize acetylcholine. It is not known how much acetylcholine is actually released during the discharge. But the amount which can be split during the duration of a discharge may give an indication of the amount possibly involved in the process, if we assume that acetylcholine is released during that period at a rate which is of similar order of magnitude to that at which it can be split. The duration of a single discharge is about 3 milliseconds. 1 gm of electric tissue can split about  $1 \mu\text{g}$  of acetylcholine during that period, that is  $5 \times 10^{-6}$  millimoles. But it is not probable that this whole amount is actually released since it is reasonable to assume that the enzyme which has to remove the active compound is present in excess. The amount of phosphocreatine split per gm and impulse is about  $0.7 \mu\text{g}$ , or  $3 \times 10^{-6}$  millimoles. Thus the amounts of acetylcholine and phosphocreatine metabolized per gm and impulse seem to be of the same order of magnitude even if we assume that choline esterase is present in a concentration of about 2-5 times in excess of that necessary to remove the acetylcholine released at a sufficiently high rate. This suggests that acetylation of choline occurs by means of an endothermic phosphorylation. Evidence showing that it is really so will be offered in the following paper.

The formation of lactic acid indicates that the phosphocreatine split may be partly resynthesized by the "Parnasreaction," i.e. by the transfer of P from phosphopyruvic acid, ATP acting again as intermediate. The lactic acid formation is however small and the greatest part of the energy required for the synthesis of phosphocreatine may be derived from the oxidation of pyruvic acid (or glucose).

Thus the following chain of reactions started by the breakdown of ACh, may be visualized (Fig. 1). ATP yields one phosphate for the resynthesis

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2 The amounts of acetylcholine and phosphocreatine metabolized as result of the discharge are of the same order of magnitude. This suggests that the energy of phosphate bonds is used for the resynthesis of acetylcholine.

3 The discharge leads also to lactic acid formation supplying an energy of  $16.8 \times 10^{-6}$  g calories per gm and impulse. The chain of reactions supplying the energy required to restore the resting condition of the electric organ thus appears to be fundamentally identical with those which are the source of energy in muscle contraction.

The electric scaling circuit was constructed in the Physical Laboratory of New York University by Dr. M. Vertner Brown of the Department of Physics of the College of the City of New York. We wish to express our thanks for the use of it.

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## RESULTS

According to Quastel and his associates acetylcholine is formed in brain slices under *aerobic* conditions only and if glucose or pyruvate is present (7). For the study of the mechanisms of enzyme reactions cell free extracts have to be used, as has been shown repeatedly. Experiments are presented in this paper in which ACh synthesis has been demonstrated in cell free extracts under *anaerobic* conditions. The mechanism involved as well as some of the properties of the newly isolated enzyme will be described in the following.

*A. Minced or homogenized tissue.* Formation of ACh in presence of adenosinetriphosphate (ATP) under anaerobic conditions was at first tested with

*Table 1* Formation of acetylcholine (ACh) in finely minced or homogenized tissue, suspended in Ringer-bicarbonate solution in  $\text{CO}_2\text{-N}_2$  atmosphere, in presence of adenosinetriphosphate. Choline chloride, sodium acetate and eserine sulfate were added

Exp No	Tissue	Kind of preparation	Addition	Duration min	$\mu\text{g}$ ACh formed		
					per vessel	per gm	per gm /hr
1	frog's brain	finely minced	—	85	6 0	40 0	28 0
2	electric organ	finely minced	—	90	12 7	86 0	57 5
3	frog's brain	finely minced	—	100	2 0	14 6	8 7
4	frog's brain	homogenized	—	85	0	0	0
			fluoride $12 \times 10^{-3}$ M	85	19 2	135 0	95 0
5	rat's brain	minced	fluoride $25 \times 10^{-3}$ M	80	0	0	0
		homogenized		80	18 3	61 0	46 0

*finely minced tissue.* The tissue was suspended in 3 0 cc frog's Ringer-bicarbonate solution in Warburg vessels. Two frog brains were used per vessel. The following additions were made: 1 2 mg of choline chloride in 0 1 cc, 1 5 mg of sodium acetate in 0 1 cc and 0 6 mg eserine sulfate in 0 1 cc. 0 2–0 3 cc ATP, containing about 0 5 mg of ATP- $\text{P}_2\text{O}_5$ , were put into the side bulb. The vessel was then filled with gas mixture (5 per cent  $\text{CO}_2$  and 95 per cent  $\text{N}_2$ ). After thermoequilibrium was reached, ATP was added and the vessels were shaken for 80–100 min. A control was run without ATP.

The results were irregular. Some were negative, a few positive. Two of the experiments with finely minced frog's brain in which ACh formation was observed are recorded in Table 1. One experiment was made with finely minced electric organ. The rate of synthesis observed was higher than in frog's brain. The substance formed could have been phosphorylcholine. This

# THE FORMATION OF ACETYLCHOLINE A NEW ENZYME· "CHOLINE ACETYLASE"

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## INTRODUCTION

THE OBSERVATIONS described in the preceding paper (8) make it probable that the breakdown of phosphocreatine supplies the energy required for the restoration of the resting condition of the nerve membrane, adenosinetriphosphate being the intermediate link. Since the alterations at the neuronal surface during activity are almost certainly connected with release and inactivation of acetylcholine (ACh), and since the amounts of ACh and phosphocreatine which are metabolized as result of the action potential are of the same order of magnitude, it was suggested that energy rich phosphate bonds are used for the resynthesis of ACh. In this paper evidence is offered which shows that this assumption is correct.

## METHODS

For the determination of ACh the method of Chang and Gaddum (3) was used in which the contraction of the frog's rectus abdominis in response to a known amount of ACh is compared with that produced by the solution with an unknown concentration of ACh. The muscle is suspended in a tube containing 80 cc. of frog's Ringer with  $\text{NaHCO}_3$  in 0.0025 M concentration. Oxygen was continuously bubbling through. If the room temperature rose above  $23^\circ\text{C}$ , cooling was applied with water running through a glass cylinder and surrounding the tube which contains the muscle. The eserine concentration was  $2.5 \times 10^{-4}$  M. The rectus of the frogs used (*Rana pipiens*) is sensitive to 0.1–0.2  $\mu\text{g}$  of ACh in 80 cc. However, 0.5  $\mu\text{g}$  is near the optimal range, the curve drawn by the lever in two min. with this amount being about 4–5 cm. high. The determinations were therefore made mostly with the latter amount as standard. 0.4 and 0.6  $\mu\text{g}$  of ACh gave distinctly different contractions, generally 2.5 mm. more or less respectively. Occasionally the muscle was less sensitive and higher amounts of ACh were used, up to 1  $\mu\text{g}$ .

The solution in which the ACh had to be determined was prepared in the same way as by Quastel and his associates (7). 2.1 cc. were taken from the solution with the tissue suspension or from the extract in which the ACh formation was being studied. 0.5 cc. of 0.1 M phosphate buffer of pH 7.4 and 0.2 cc. of  $\text{NHCl}$  were added. The pH drops hereby to 2–3. After about 30 min. the solution was neutralized by addition of 0.2 cc. of  $\text{N NaOH}$ , centrifuged and the supernatant fluid was used for the test.

The solution to be tested was generally added in amounts of 0.1–0.2 cc. and sometimes for control in amounts of 0.3–0.4 cc. In such a small volume the interference of other substances, especially choline and potassium in higher concentration, was shown by repeated controls to be negligible. Only if inhibitory effects were expected higher amounts of the solution to be tested were used, 0.5 cc. or even more. A control was always run with the same amount of extract to which no adenosinetriphosphate (ATP) was added. ATP was prepared according to the method of Lohmann (6). Several modifications, however, were applied which facilitate the preparation and improve the yield.†

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‡ We are greatly obliged to Dr. Severo Ochoa who suggested the modifications.



*C Some properties of choline acetylase — Stability* The enzyme kept in the refrigerator loses in 24 hours about 50–80 per cent of its activity Freezing did in some cases destroy the enzyme, in other cases the activity remained unchanged The reason for this irregular behavior has not yet been found

*Influence of pH* Two observations were made so far, in one experiment with cat brain, the pH was changed by means of different phosphate buffers

Table 2 Formation of ACh in brain extracts in presence of adenosinetriphosphate Choline chloride, sodium acetate, sodium fluoride and eserine sulfate were added Duration 90 min

Exp No	tissue	μg ACh formed		
		per vessel	per gm	per gm /hr
1	rat brain	12 0	39 4	26 3
2	rat brain	52 0	150 0	100 0
3	rat brain	34 0	93 0	62 0
		34 0	93 0	62 0
4	rat brain	18 8	56 2	37 5
5	rat brain	40 0	105 0	70 0
6	rat brain	22 0	53 0	35 5
7	guinea-pig brain	11 2	27 7	18 5
8	pigeon brain	27 6	69 0	46 0
9	cat brain	11 1	17 1	11 4
10	cat brain	11 1	17 1	11 4
11	rat muscle	0	0	0
12	rat liver	0	0	0

In three different vessels the final pH was 6.6, 6.8 and 7.0 respectively, as determined by means of an electropotentiometer 17 μg of ACh were formed per vessel, independent of the pH In another experiment with rat brain the following formation of ACh was observed At pH 7.5, 12.9 μg, at pH 7.1, 13.6 μg, at pH 6.5, 11.0 μg These observations seem to indicate that the enzyme is not sensitive to variations close to pH 7.0 at which most experiments were carried out

*Effect of K<sup>+</sup>, NH<sub>4</sub>Cl and HCN* Quastel and his associates (17) found that ACh formation in brain slices in oxygen is increased by addition of K<sup>+</sup> When 0.027 M K<sup>+</sup> was added, the effect was optimal The authors assumed that K<sup>+</sup> may act by increasing the cell permeability No effect of K<sup>+</sup> was

intermediate, however, does not affect the frog's rectus (2) Moreover, it withstands boiling with  $N$  NaOH at  $100^{\circ}\text{C}$  for several hours (10) Addition of  $N$  NaOH completely destroys the substance formed in our experiments within 1 min, whereas boiling with weak acid, at pH 5.0, does not change the substance, as should be expected if the compound formed is ACh

Since ATP does not penetrate the intact cell the possibility was explored whether ACh formation could be obtained in homogenized tissue in presence of ATP In this case, however, ATP is rapidly split by adenosinetriphosphatase Ochoa observed that fluoride inhibits adenosinetriphosphatase, but not the transfer of phosphate from ATP to a phosphate acceptor (9) If fluoride was added to the homogenized tissue a high rate of ACh formation occurred Without fluoride no ACh was found (exp No 4) In presence of fluoride, synthesis was also observed in homogenized rat brain as shown in exp No 5, but not in minced tissue

Since ACh formation occurred even after destruction of the cell, the experiments suggested that it might be possible to extract from the cell the enzyme performing the synthesis

*B Extraction of an acetylcholine forming enzyme from brain* Extracts were prepared in the following way 2-4 rat brains were cooled on ice, then ground with an homogenizer During the grinding the tube was continuously kept in ice 5-10 cc of the solution were taken per brain containing KCl in 0.03  $M$  concentration Either sodium bicarbonate or phosphate were used as buffer in a concentration usually not higher than 0.01  $M$  Sodium acetate, choline chloride, eserine sulfate and ATP were added at the same concentration as described above Sodium fluoride was always added, the final concentration being  $25 \times 10^{-3} M$  2.5-3.0 cc of extract were used per vessel so that the final volume was between 3 and 4 cc ACh formation was regularly obtained The results are summarized in Table 2 The most active tissue examined was rat brain 35-100  $\mu\text{g}$  of ACh were formed per gm an hour The lower value in exp No 1 may be due to the use of Ringer since Ca activates adenosinetriphosphatase Cat and guinea-pig brain appeared to be less active No activity was formed in liver and muscle extracts

Presence of choline, eserine and fluoride appears necessary for an optimal rate of synthesis under the conditions used If, on the other hand, no acetate was added, the rate was still the same or but slightly decreased The following figures may be given as an example In exp No 3 (Table 2) in which 62  $\mu\text{g}$  of ACh were formed per gm an hour with all additions, no ACh was formed without eserine, only 9.6  $\mu\text{g}$  without fluoride, which is a decrease of 85 per cent Without choline 20.0  $\mu\text{g}$  formed, a decrease of 68 per cent Without acetate 66.6  $\mu\text{g}$  were found which is nearly identical to the value obtained with acetate

The experiments show that brain and nerve tissue (electric organ) contain an enzyme which can be extracted and which in presence of the free energy of ATP synthesizes ACh The enzyme will be called "choline acetylase"

of a great variety of enzymes contains them Iodoacetic acid (I A A ) acts specifically with  $-SH$  groups as was shown by Rapkine (10, 11) and Dickens (4) ACh formation is strongly inhibited by sodium iodoacetate One typical experiment is given in Table 3, No 4 If the extract is shaken for 20 min with a  $1 \times 10^{-3} M$  solution of I A A the inhibition was already 58 per cent With longer action of the compound on the enzyme a stronger inhibition was obtained In another experiment the inhibition was 83 per cent with  $5 \times 10^{-4} M$  concentration of I A A having reacted with the enzyme during 120 min , and 100 per cent with a  $2 \times 10^{-3} M$  concentration shaken for the same period

It is possible that pyruvic acid is the precursor of acetic acid in the acetylation process Since, therefore, inhibition of pyruvic acid formation by I A A could be responsible for the inhibitory effect on ACh formation, pyruvate was added to the extract besides I A A No effect of pyruvate was observed, the inhibition of ACh formation being as complete as without pyruvate (exp No 2) Sulfhydryl groups are highly sensitive to Cu Choline acetylase is practically completely inhibited by a  $3 \times 10^{-5} M$  concentration of Cu and the inhibition was still 66 per cent at a  $1.5 \times 10^{-5} M$  concentration

Iodine, which easily oxidizes  $-SH$  groups, had a marked inhibitory effect at  $1 \times 10^{-4} M$  concentration, if allowed to act on the enzyme for 40–60 min An example is given in exp No 2 These observations make it possible to assume that choline acetylase may belong to the enzymes containing sulfhydryl groups However, more evidence is desirable to support this assumption

### DISCUSSION

Evidence is offered that an enzyme can be extracted from brain and nervous tissue (electric organ)—and apparently only from those—which synthesizes ACh in presence of ATP It is generally accepted that ATP is the primary source of energy in muscle contraction Engelhardt (5) recently made observations from which he concluded that this compound may be directly connected with the mechanical change occurring during muscular contraction Direct evidence, however, is still lacking and the question whether contraction or relaxation of the muscle is linked to the effect of ATP is still open The propagation of the nerve impulse is connected with electrical and not with mechanical changes Strong evidence has accumulated that these electrical changes are connected with formation of ACh The preceding paper indicates that energy rich phosphate bonds can supply the energy required by the action potential The present paper shows that the free energy of ATP can in fact perform ACh synthesis as suggested in the "acetylcholine cycle"

The question still remains open what the phosphorylated intermediate compound is Theoretically it could be acetylphosphate or phosphorylcholine exchanging the phosphate for choline or acetate resp In this connection it may be of interest to know what the precursor of acetate is There are several

Table 3 Effect of  $\text{NH}_4^+$ ,  $\text{Cu}^{++}$ , iodine and iodoacetic acid (I.A.A) on the activity of choline acetylase Conditions as those in the experiments recorded in Table 2

Exp No	Compound added	Concentr M	time min.*	$\mu\text{g}$ ACh formed per vessel	Inhib per cent
1	Control			40 0	
	Cu	$16 \times 10^{-3}$ $3 \times 10^{-3}$		0 2 5	100 94
	$\text{NH}_4\text{Cl}$	$2 \times 10^{-3}$		40 0	0
		$4 \times 10^{-3}$		35 0	12
		$8 \times 10^{-3}$		40 0	0
2	Control			13 5	
	I.A.A	$1 \times 10^{-3}$	20	0	100
	I A A +pyruvate	$1 \times 10^{-3}$ $3 \times 10^{-3}$	20	0	100
	Iodine	$1 \times 10^{-4}$	45	3 4	75
3	Control			22 0	-
	Cu	$1.5 \times 10^{-3}$		7 5	66
	I.A.A	$1 \times 10^{-3}$	60	0	100
4	Control			29 0	
	I A A	$2 \times 10^{-4}$	120	17 0	41
		$1 \times 10^{-3}$	20	12 2	58
		$1 \times 10^{-3}$	60	9 5	68
		$1 \times 10^{-3}$	120	5 1	83
		$2 \times 10^{-3}$	20	7 6	74

\* Time of addition of compound before addition of ATP

observed at a concentration varying from  $0.02$ – $0.06$  M This seems to indicate that the effect observed by Quastel is not directly connected with ACh formation but may be due—as assumed—to a permeability change

Quastel *et al* found under their experimental conditions a strong inhibitory effect of  $\text{NH}_4\text{Cl}$  on ACh formation at a concentration of  $0.05$  M They concluded that  $\text{NH}_4^+$ , since it, like  $\text{K}^+$  causes an increased permeability, has a direct inhibitory action on ACh synthesis In extracts however, no inhibitory action was found as shown in exp No 1 of Table 3 when concentrations of  $\text{NH}_4\text{Cl}$  were used between  $0.02$  and  $0.08$  M No effect was observed with cyanide in a concentration of  $12 \times 10^{-3}$  M and  $4 \times 10^{-3}$  M This makes it improbable that choline acetylase contains an active metal group

*Iodoacetic acid* Barron (1), recently reviewing the enzymes containing sulphydryl groups, stressed the significance of these groups since the protein



possibilities which may be studied on dialyzed extracts. Many other problems remain open which require further investigations.

### SUMMARY

An enzyme has been extracted from brain and nervous tissue (electric organ) which forms acetylcholine. The formation occurs only in presence of adenosinetriphosphate (ATP). The enzyme is called choline acetylase.

The formation of ACh is greatly enhanced by fluoride which, according to Ochoa, inhibits adenosinetriphosphatase but not the transfer of phosphate to a phosphate acceptor.

$K^+$  at a concentration between  $2$  and  $6 \times 10^{-2} M$  and  $NH_4^+$  at a concentration between  $2$  and  $8 \times 10^{-2} M$  do not affect the enzyme. Cu, iodoacetic acid and iodine have a strongly inhibitory effect. The implications of these observations for the mechanism of nerve activity are discussed.

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The ablations were made aseptically, those from the cortex with a sharp-edged metal spatula and those of subcortical nuclei by gentle suction through a long-nosed glass sucker. The thalamus was reached after section of the corpus callosum, the hypothalamus from the base of the brain, approached rostrally above the optic chiasm. Various approaches to the

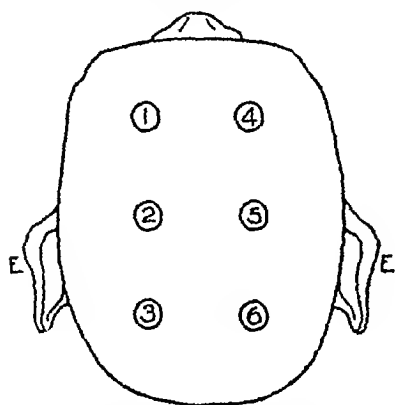


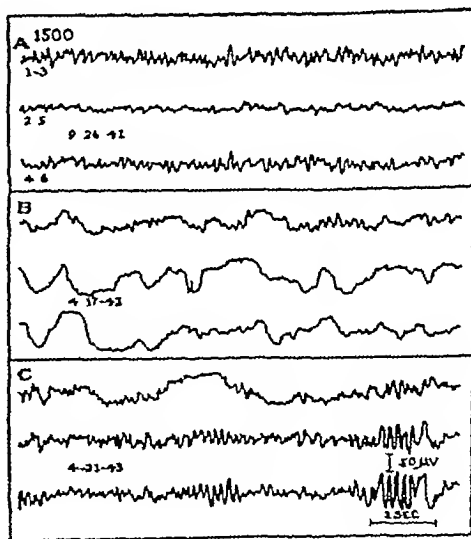
FIG 1 Diagram of monkey's head showing positions in which EEG leads were applied

basal ganglia were used as described in the protocols. The 52 monkeys used were either *Macaca mulatta* or mangabey (*Cercocebus torquatus atys*). Of these 14 were operated upon in infancy.

#### EXPERIMENTAL DATA

With one exception abnormal EEGs always appeared in animals with definite symptoms of central nervous system deficit, and the severity of the

FIG 2 Changes in EEG resulting from ablations of areas 6, 8 and caudate nucleus. A, normal record B, after extirpations, left on Dec 17, 1941, and right on Apr 16, 1942, upper tracing (lt) shows a burst of high amplitude, lower tracings show irregular slow waves, as result of operation on preceding day C, one year later, characteristic bursts of high amplitude present in all leads



EEG disturbance was usually commensurate with the severity of the symptoms. Both were thus directly dependent on the size of the lesions within the basal ganglia since it has been shown (8) that there is little or no localization within the nuclei of the basal ganglia and that hence small lesions are im-

# EFFECTS ON EEG OF CHRONIC LESIONS OF BASAL GANGLIA, THALAMUS AND HYPOTHALAMUS OF MONKEYS\*

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IN A PREVIOUS COMMUNICATION (10) it was stated that injury to the cerebral cortical tissue of monkeys had no significant effect on the electroencephalogram (EEG), but that combined lesions of cortex and basal ganglia caused prompt and often permanent alterations in the pattern of cortical potentials. In the present paper it will be demonstrated that lesions restricted to thalamus and hypothalamus also have an effect on EEG in chronic conditions. In *acute* preparations like effects have been recorded by numerous investigators. Dusser de Barenne, Garol and McCulloch (4) produced changes in EEGs of monkeys by lesions in caudate nucleus and Dusser de Barenne and McCulloch (5) found changes in the postcentral cortical electrogram after lesions of the corresponding sensory nuclei of the thalamus and changes of EEG after strychninization of these postcentral areas. Dempsey and Morison (3) and Morison and Dempsey (11) similarly found focal changes in the parietal area after lesions of the thalamus of monkeys, and Obrador (12) abolished the EEG of cats by destruction of the hypothalamus. Acute decortication of monkeys under dial anesthesia, furthermore, changes the pattern of electrical potentials from basal ganglia and destruction within thalamus or hypothalamus slows the rate and lowers the amplitude of EEGs obtained from the surface of the cortex (7).

The present observations have been made on 52 chronic monkeys over a period of days, weeks or months before and after lesions of the subcortical nuclei. Such lesions have been made individually in the several nuclei or in combinations and with or without additional cortical ablations. Observations on the functional and anatomical changes of these animals will be published elsewhere (8).

## METHOD

A Grass ink-writing 3-channel oscillograph was used. Records were taken by means of electrodes fastened to the skin of the scalp as described in a previous article (9). The same six combinations of leads from each side of the head in frontal, mid-parietal and occipital positions were used, but records from only three of these positions have been reproduced here as experience has shown that these three are the best for demonstrating both focal and general changes in the EEG following isolated ablations. The positions are shown in Fig. 1. The numerals for each position are then given in the succeeding figures which are reproductions of strips of EEG records. As will be seen, the leads 1-3 represent potentials obtained from the length of the left hemisphere, leads 4-6 are in similar position on the right, and leads 2-5 give intra-hemispherical potentials.

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there is much more irregularity and some slowing of rate. Six months later, in C, the record shows extreme "fencing" or hypersynchrony, as it had throughout the intervening time. Ten days before the record in D, the right ansa lenticularis was sectioned producing much more marked irregularity although the "fence" arrangement and the absence of fast waves persists. This last finding occurred in all five animals in which the ansa lenticularis was sectioned either unilaterally or bilaterally.

Lesions anywhere in the basal ganglia if they were large enough produced the same effect as those shown in Fig. 2 and 3. The irregular bursts of high amplitude, regular potentials are characteristic of such disturbances. There

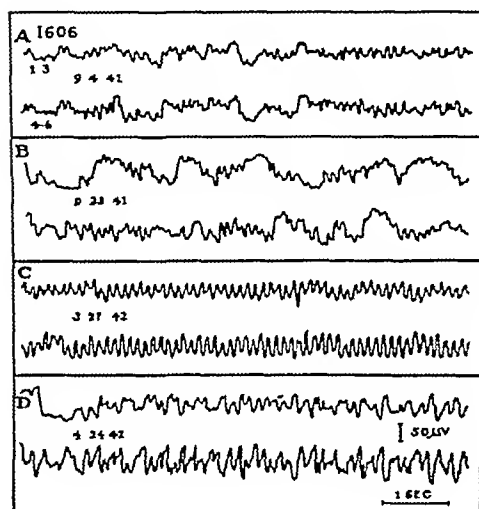


FIG. 3. Effect of ablation of area 8 and caudate (B and C), and of section of ansa lenticularis (D). Note regular high-amplitude rhythm in C and irregularities in D (see text).

was a suggestion that hypersynchrony was more intense following lesions in which caudate was injured and that irregularity of pattern and large, high amplitude waves were found more often from injury to ansa or globus pallidus. Often, however, it would be impossible to distinguish between the records of these three lesions.

Figure 4 has excerpts from the records of the left and right hemispheres of a monkey from which portions of the putamen and globus pallidus were removed from an approach via the base of the brain, the left on Feb. 22, 1943 and the right on March 22, 1943. The normal record is given in A. In B, three days after the first operation, the left, operated side has waves of low amplitude and slow, those of the right are higher and somewhat irregular. Six days later in C when the effect of operation has worn off, both sides show slowing and definite irregularity but the rate on the right is still faster than that on the left. In D, 12 days after the second operation, there is a characteristic picture, many slow high-amplitude waves appear in both sides. The lesion at autopsy is shown in Fig. 6.

mediately compensated for by the remaining tissue both ipsilateral and contralateral to the injured area. Small lesions of caudate or putamen even if bilateral caused no alteration in EEG and this was true also in thalamus and hypothalamus. Although all subtotal lesions were followed by some recovery, large bilateral lesions resulted in permanent changes in EEG, or at least those which lasted more than a year after operation (Fig. 2).

The exception to the rule that size of lesion directly influenced degree and duration of both symptoms and changes in EEG was demonstrated in the 14 animals operated on in infancy. Such infants always showed minor symptoms as compared to older animals. But they had the most extreme and enduring changes in the EEG. Many of these, kept for two or more years after operation, grew to the size of others operated on later in life. They did not however develop severe symptoms of motor deficit although their EEGs remained very abnormal.

*A Lesions of basal ganglia.* The observation made in a preliminary paper (10), that combined ablations from area 6 and caudate nucleus produced marked and permanent changes in EEG has been confirmed by the addition of more animals with similar ablations (11 in all). Figure 2 illustrates such changes. In A a normal preoperative record is given in which both medium 8 per second rhythm and fast waves of about 20 per sec can be seen. On Dec. 17, 1941 the left areas 6, 8 and caudate head were removed. On April 16, 1942 the same areas were extirpated from the right side. Tracing B made the day after this second operation shows the characteristic changes which follow this procedure. On the upper tracing, from the left side, the rate of about 8 per sec. is normal and the shape of the waves nearly so although there is a suggestion of the periodic, high-amplitude bursts shown more definitely in C. The lower two lines of B, representing the right hemisphere and intrahemisphere potentials, show the marked slowing, unevenness and diminution in amplitude characteristic of a postoperative picture. In C, made a year later all three leads show abnormalities absolutely characteristic of injury to basal ganglia. The rate is again 8 per sec. and amplitude about that of the preoperative record. There are fewer of the fast waves, however, while the 8 per sec. medium waves are more regular and have periodic bursts of high-amplitude pointed waves like those of hypersynchrony in the human. In this animal, a mangabey of medium size, records were taken frequently for almost two years. During the postoperative period the only change was in the rate, which was slowed by the operation and then returned to preoperative levels. This animal has been rigid and has marked tremor which is unabated now more than a year after its last operation.

Figure 3 shows similar changes from the records of a mangabey which had had caudate and putamen removed on June 23, 1941, the left area 8, on September 15, 1941 the same areas from the right, and on April 13, 1942, section of the right ansa lenticularis. In A, the unilateral removal of cortex, caudate and putamen has done little to the EEG unless a certain amount of irregularity is present. In B, within the week after the second operation

ously that such small lesions of thalamus as of basal ganglia caused no discernible effect on the EEG obtained from the surface of the scalp

Figure 5, A, B, and C show the changes which occur following such thalamic lesions. On Oct 26, 1942 a large excavation was made from the thalamus after section of the overlying corpus callosum. On January 22, 1943 the hypothalamus was nearly completely destroyed. The normal clearly defined

10 per sec waves seen in A have changed to slow wavering irregular rhythm in B ten days after the lesion was made in the thalamus. Three months later, the rate has increased to about 8 per sec, which is not quite that of the preoperative record, but there was still marked irregularity and a wavering baseline. This irregularity and tendency to change was probably the most noticeable characteristic of the monkeys with thalamic injury. There was always, in addition, a slowing of rate, and frequent appearance of large slow hill-like waves such as are shown in both B and C.

*C Lesions of hypothalamus*  
Records from animals with lesions of the hypothalamus have been made in five cases, and in each, the changes were similar, although the clinical symptoms varied. Two of

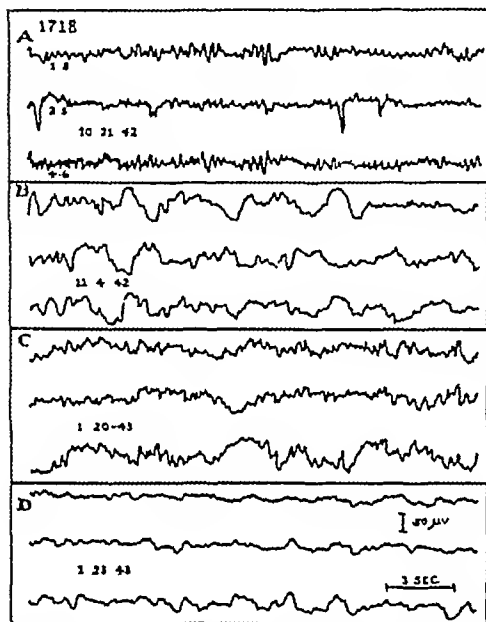
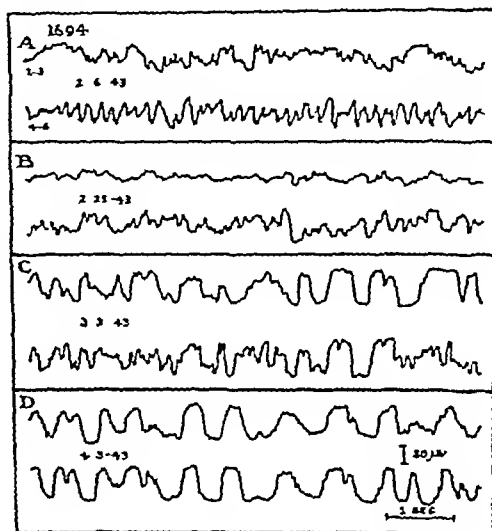


FIG 5 Effect of lesions of thalamus (B and C) and hypothalamus (D) (see text)

these, one of which was the last described animal above, which had already had a lesion of the thalamus, developed, after the hypothalamic ablation, a profound "pathological" sleep such as has been described by Ranson (14) in monkeys. These two (Fig 5D and 7) had the most pronounced changes in EEG, the other three had changes of the same nature but of less severity. In all the amplitude of EEG diminished almost to zero, the rate slowed and almost nothing could be found which resembled the normal EEG pattern. In contrast sleep in these monkeys, either normal or when induced by luminal causes the same changes as in man, and which are the opposite of those which followed hypothalamic injury. In sleep the amplitude becomes greater, the rate more variable, but often very fast, and the entire picture is one of accentuation of the normal alpha rhythm. In Fig 7 is shown the record of one of these somnolent monkeys five days after the lesion was made which is shown in Fig 8, and three days before its death from hypostatic pneumonia, the result probably of its extreme inertia.

The tendency to irregularity and variability of pattern which was present in these animals with lesions of the basal ganglia was demonstrated in another way, namely by the occurrence of epilepsy after operation in a great many cases. It happens occasionally that, following operation upon the motor cortex, epilepsy appears during the first postoperative week but this is extremely rare. It was a common symptom when the basal ganglia together with cortex were injured. It appeared less often when basal ganglia alone were damaged. Since it was periodic and transient there are no valid statistics

FIG 4 Changes in EEG following ablation from left (B) and right (C) putamen and globus pallidus (see text)



as to its incidence, for clinical epilepsy was seen in a number of animals at times when no record was taken, and may have been missed in as many more. On the other hand, sub-clinical seizures were not infrequently recorded in animals which had never been seen to have grand mal attacks and appeared on the records of some animals which had been known to have attacks but which at the time of the EEG seizure, appeared normal clinically. All this epilepsy occurred within a few weeks after operation and disappeared then, never to reappear. It is probable therefore that it had to do with tension and edema of the tissues which were disturbed at the time of operation. The reason for its high incidence in combined cortical-subcortical lesions and not in those limited to cortex may be due merely to this edema, for there was, of necessity, more distortion of motor pathways in the combined cortical and subcortical lesions.

**B Lesions of thalamus** Lesions of the thalamus were made in five monkeys. In each, the approach was via a sectioned corpus callosum. In each large lesions were made involving either the entire massa intermedia (1 case) or most of one side of the thalamus (3 cases). One animal had bilateral ablations from this region. No attempt was made to produce small lesions restricted to one or more nuclei in this series because it had been found previ-

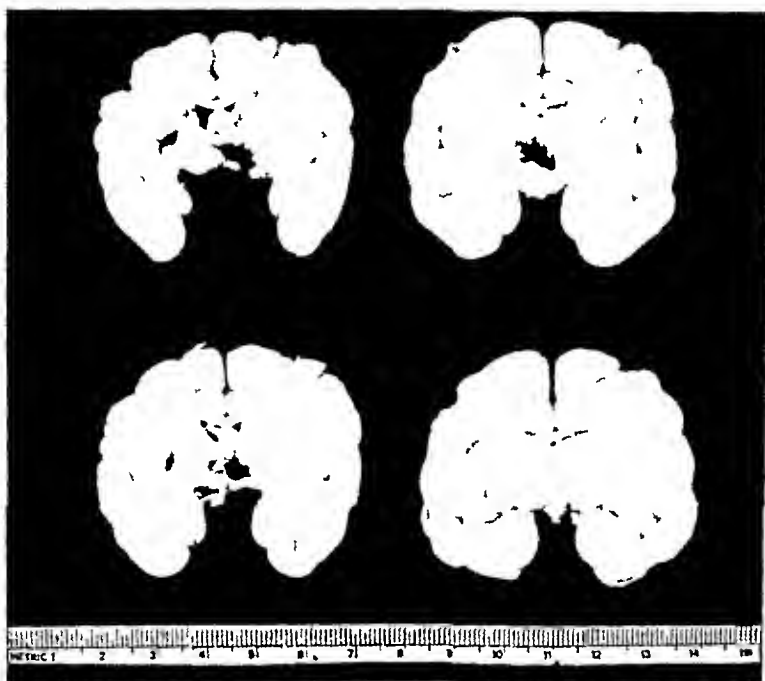


FIG 8 Lesion of hypothalamus producing EEG record shown in Fig 7

### DISCUSSION

The absence of abnormal EEGs in monkeys after lesions restricted to the cerebral cortex, although they appear when the sub-cortical nuclei have been injured is not consistent with the usual present-day concept of the EEG which has been shown both clinically and experimentally to be directly concerned with cortical excitability. Nevertheless there is abundant recent evidence which relates the subcortical areas as well to the EEG.

The absence of changes from cortical injury in monkeys can be explained by several factors. First, the size of the monkey's head is so small, that when obtaining records from the scalp and not directly from the surface of the brain no localization, or at best only lateralization appears. Since in man, nearly all clinical localization today is made by comparing the EEG from one area with that of another (2, 6) and this factor is not useable in the monkey small changes could not be detected in the latter. To this must be added the fact that the "normal" EEG even in man is only the usual for that patient as compared to what have already been established as standards and that the material for standards in the monkey at present is much less. That acute changes of record can actually be obtained in monkeys by cortical excitation is well known, and Pacella, Barrera and Kopeloff (13) have shown that chronic irritation of the cortex can produce recordable episodic seizures. This



FIG 6 Bilateral lesions of putamen and globus pallidus of monkey, whose record is shown in Fig 4 (No 1694)

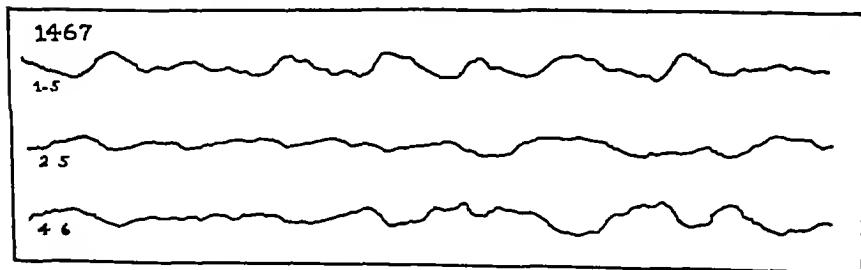


FIG 7 EEG of somnolent monkey with lesion of hypothalamus shown in Fig 8

condition and that they resemble certain dysrhythmias found in epileptics between attacks and also those found following acute head injury

Buchanan, Walker and Case (1), confirm these observations and stress the similarity between the changes in epilepsy and in chorea. They consider that the families of children with chorea, like those with epilepsy, show changes which are of the same nature although less pronounced than those of the patients. It is possible that the changes found in both man and monkey following acute head injury may be dependent on injury or distortion of the basal ganglia also. Evidence at present is strongly in this direction. For, neither in man nor monkey can the changes which occur acutely in the two conditions be told apart. It is well known also that the basal ganglia are particularly susceptible to minute petechial hemorrhage following trauma which might well be the underlying cause of the disturbance.

The relation of the hypothalamus to sleep has long been known but its exact influence on and interconnection with the cortex are still not clear. Both the work of Obrador (12) and ours on acute preparations (7) is borne out by the present findings that destruction of the hypothalamus diminishes or abolishes the pattern of cortical potentials. How this relates to the apparent "release of function" exerted by the normal hypothalamus during sleep is difficult to imagine.

#### SUMMARY

1 In monkeys chronic lesions of the subcortical nuclei have been found to produce changes in the EEG although lesions restricted to cortical tissue cause no such change.

2 Lesions of the basal ganglia if large enough, or of basal ganglia and cerebral cortex cause permanent alteration in the EEG.

3 Epilepsy, either clinical or subclinical and detectable by EEG, was a frequent finding following lesions to basal ganglia.

4 The changes of EEG following lesions of basal ganglia can be directly correlated with the functional changes in the monkey and are similar to those seen in human children with chorea.

5 Lesions of the thalamus caused marked slowing of rate, irregularity of pattern and the appearance of high, slow rounded waves at frequent intervals.

6 Lesions of the hypothalamus caused great slowing of the rate and diminution of amplitude. With large destruction practically no pattern of potentials remained.

7 This is in direct contrast to the effects of sleep which are to increase amplitude and intensify the normal pattern.

8 It is suggested that the post-traumatic changes which appear in both man and monkey may be directly related to changes within the basal ganglia.

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was done by placing irritating substances on the surface of the cortex. Apparently the reasonably clean-cut extirpation has no such irritative effect.

There is recent clinical evidence that primary subcortical lesions may be the cause of epilepsy and hence of abnormal EEGs in man. Foerster and Penfield (6) have discussed what has since become a well-recognized clinical fact, that trauma to the brain, when deep, as in penetrating wounds, frequently produces epilepsy as a late manifestation and that this is due to traction on the cortex by the formation of scar tissue. Since there can be no doubt that epilepsy is a manifestation of cerebral cortical irritation, and since it was noticeably frequent in these animals with lesions of the basal ganglia, it is probable that disturbance of the cortico-subcortical pathways from the motor area with consequent pressure or traction on the excitable motor cells must have been at the basis of the epileptic attacks and of at least some of the abnormal records which were not accompanied by seizure.

It is noteworthy, however, that lesions of neither thalamus nor hypothalamus caused epilepsy but several additional facts must be considered in this respect. First, it happened that the ablations of these latter two groups did not have concomitant lesions of cortex as did many of those of the basal ganglia which produced epilepsy, second, neither thalamus nor hypothalamus are so near the internal capsule and corticospinal tracts as are the basal ganglia, third, since the basal ganglia are functionally more intimately related to motor performance this may be the reason that their disturbance produces epilepsy, fourth, the caudate nucleus, at least, is known to be directly related to the suppressor function of area 4s of the cortex (4) and if removal of suppression is one cause of epilepsy this may conceivably play a part in the type of abnormal EEG which was found. Distortion of the subcortical tracts would be as likely to appear after thalamic and hypothalamic lesions as after those of basal ganglia for, in our experience in the animals that came to autopsy some days after operation, there was as apt to be edema of the tissues in one lot as in the other.

The presence of abnormal EEGs in patients with chorea has been now established. In two recent articles (1, 15) the changes described in the EEGs of a series of children with Sydenham's chorea, which is known to be a disease affecting basal ganglia primarily, are strikingly like those found in the present series.

Both groups of authors stress the constancy of abnormal EEGs in this condition and furthermore the relation, both clinically and from electroencephalographic evidence, between chorea and epilepsy. The children of the series of Usher and Jasper all had generalized abnormalities of EEG, consisting of a decrease or absence of the alpha rhythm and presence of continuous slow delta wave activity of high amplitude. Slowing of rate and increased amplitude were the most conspicuous changes in the monkeys. In addition, the more severe the chorea the more gross the changes in EEG in the children, and improvement in EEG was always correlated with clinical recovery. The authors state that the changes in EEG of chorea are not specific for this





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# HAND AND FOOT PATTERNS OF LOW ELECTRICAL SKIN RESISTANCE: THEIR ANATOMICAL AND NEUROLOGICAL SIGNIFICANCE\*

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IN A PREVIOUS PAPER it was shown that sympathectomized areas on any part of the body can be sharply defined by means of the electrical skin resistance method (1). These areas, which do not sweat, have a higher resistance than the surrounding normal areas. In sympathectomized patients these patterns of high skin resistance, which agree closely with the distribution of the sensory dermatomes, come out most clearly after the entire body has been heated in a hot air cabinet—the temperature used being that which in the normal person causes a general decrease in skin resistance to a low level with the disappearance of all patterns. At ordinary room temperature, most normal persons manifest well defined areas of low resistance on the face, hands, feet, axillae, and antecubital fossae (2). When an individual is immersed in a hot air bath, the facial area enlarges until it includes first the entire face, then the head and neck, and finally the entire body. Both cooling and sleep cause a marked constriction to a very small area which finally includes only the lips. The area of low resistance most commonly present in normal individuals at room temperature coincides closely with the hairless area of the skin on the face of gorillas and chimpanzees and other primates. The patterns of the areas of low skin resistance coincide closely with the patterns of areas of remaining sensitivity found on the faces of patients with syringobulbia and, like them, vary concentrically around the mouth.

In the following experiments detailed examinations were made to determine the shape and distribution of the areas of low skin resistance on the hands and feet under varying conditions. Are these areas fixed or do they also vary with external temperature and sleep? Do the patterns correspond to the distribution or activity of the sweat glands or peripheral blood vessels? Do the patterns show any relation to the peripheral nerves, sensory dermatomes, or central or autonomic nervous system anatomy?

## METHODS

Previous papers contain a full description of the technique (1, 2). By means of a small ear clip one electrode, a  $\frac{1}{2}$  inch zinc disc, is clasped to the ear lobe—the skin of which has been pricked with a hypodermic needle to eliminate the resistance of the skin under this electrode. A paste made of kaolin and saturated zinc sulphate solution establishes contact between this electrode and the skin. The movable electrode, which consists of a small  $\frac{1}{2}$  inch zinc disc fastened at right angles to the end of an insulated zinc rod, is held by the operator and can thus be touched against the skin on any part of the body. No paste is used with this electrode. The dermatometer used for these experiments consists of a 4.5 V battery, a

\* Supported by a grant from the John and Mary R. Markle Foundation

microammeter, and a variable resistor. The instrument is used only to register differences in skin resistance, not to obtain actual measurements.

The following procedure is used in mapping areas of high and low skin resistance. After the fixed electrode has been attached to the ear, the instrument is set by means of the resistor so that a moderate difference in potential exists between the electrodes. As the movable electrode is pressed lightly against the skin on different parts of the body, the ammeter is watched closely for sharp changes in the amount of current flowing through the circuit. This amount depends largely on the resistance of the skin under the movable electrode. When the resistance is high, little or no current flows and the indicator on the dial does not move, when it is low, the current that flows is strong and the indicator moves quickly across the face of the dial. The surface of the skin is explored with the movable electrode until a point is found at which the resistance shows either a sharp rise or fall. This point is then marked with a black skin pencil. Adjacent areas are explored until another point of marked change in skin resistance is located. Successive points thus located are joined together with a pencil line and the process is repeated until finally areas of high and low skin resistance are completely defined, that is, until the two ends of the line joining the points come together. Seventy normal adults and ten new born infants were used in these experiments.

## RESULTS

*A Hands* Figure 1 shows the two most common patterns of the areas of low electrical skin resistance found on the palmar and dorsal surfaces of the hands of normal individuals. The pattern shown in Fig. 1A (stippled area) includes all of the palmar surface as well as the dorsal surface of the two distal phalanges of the fingers and thumb. The patterns shown in Fig. 1B include most all of the palmar surface but none of the dorsal surface.

### NORMALS AREAS OF LOW ELECTRICAL SKIN RESISTANCE

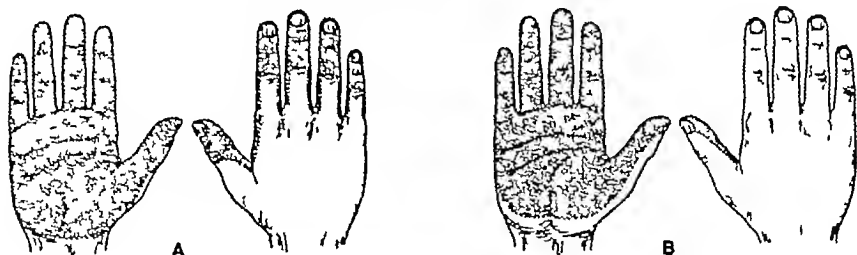


FIG 1

The resistance on the outside of the closed area was usually about 4 times higher than on the inside, while the line of separation between the areas of high and low resistance was scarcely more than  $\frac{1}{8}$  of an inch in width. This line of demarcation was quite as sharp as between areas of sympathectomized and normal skin described in a previous paper.

*Effect on hand patterns of cooling and heating the body* Figure 2 gives a typical record of the effects produced on the hand pattern of low electrical skin resistance by cooling and heating the body. In these experiments the subjects were immersed to the shoulders in a continuous tub bath, the hands and arms rested on the sides of the tub and remained dry and accessible for the skin mapping. Under normal conditions, that is, at room temperature,

the pattern of low resistance of this subject included all of the palmar surface and none of the dorsal surface. After 2 minutes in a bath at 75°F the area of low resistance on the palmar surface became constricted and divided

### TEMPERATURE EXPERIMENT BODY EXCEPT FOR HANDS IMMERSED IN WATER BATH COOLING

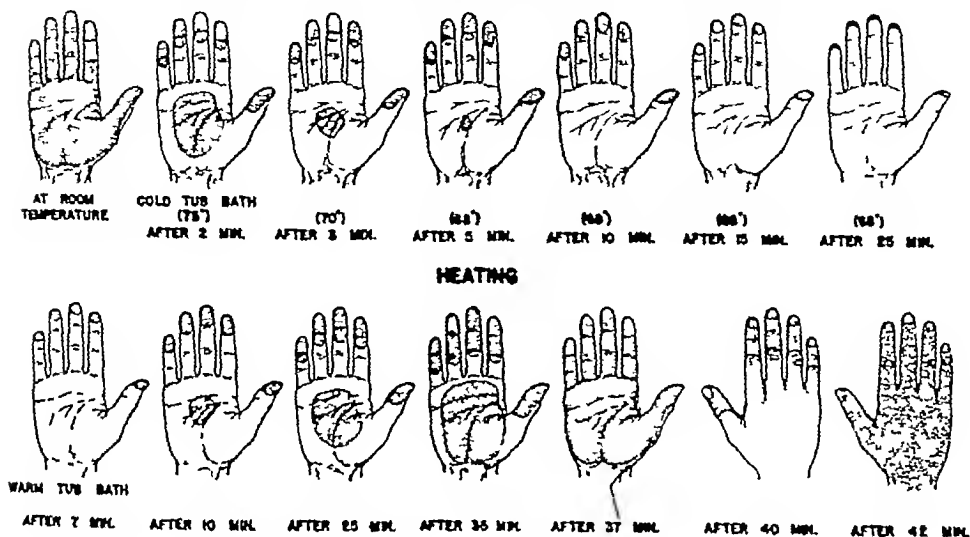


FIG 2

into small areas, one on the tip of each finger and thumb, and another, larger one, in the center of the palm. Progressively, with time and with the lowering of the temperature of the bath, these areas grew smaller until finally after 25 minutes' immersion when the bath had cooled to 68°F, they included only the tips of the fingers and thumbs. At this time hot water was run into the tub gradually.

After 7 minutes the areas on the tips of the fingers had enlarged slightly. After 10 minutes the palm of the hand again showed a small area of low resistance and the areas on the tips of the fingers had become larger. During the next 20-25 minutes the finger, thumb, and palmar areas gradually increased in size until they became consolidated again into a large area which cov-

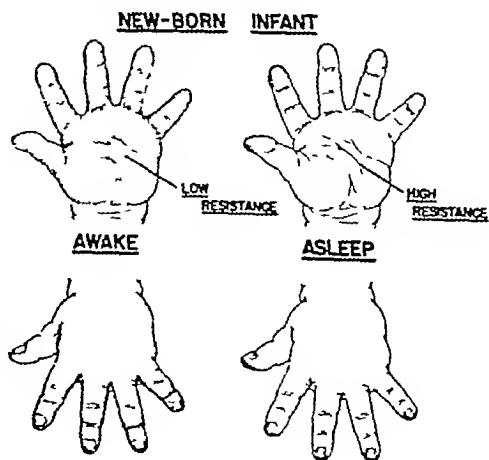


FIG 3

ered the entire palmar surface. With longer heating the area of low resistance spread further, including the dorsal surface of the fingers and thumb up to the second joint, the entire hand, then glove shaped patterns, and finally the entire arm. Ten other subjects gave essentially similar records.

*Changes in hand patterns during sleep* In a previous paper it was reported that during sleep the facial area of low skin resistance contracts markedly. In the present experiment we found that the hand patterns also become very much constricted. These observations were made on newborn infants. Figure 3 gives a typical record. During the waking state this baby's area of low resistance included the entire palmar surface and all of the dorsal surface

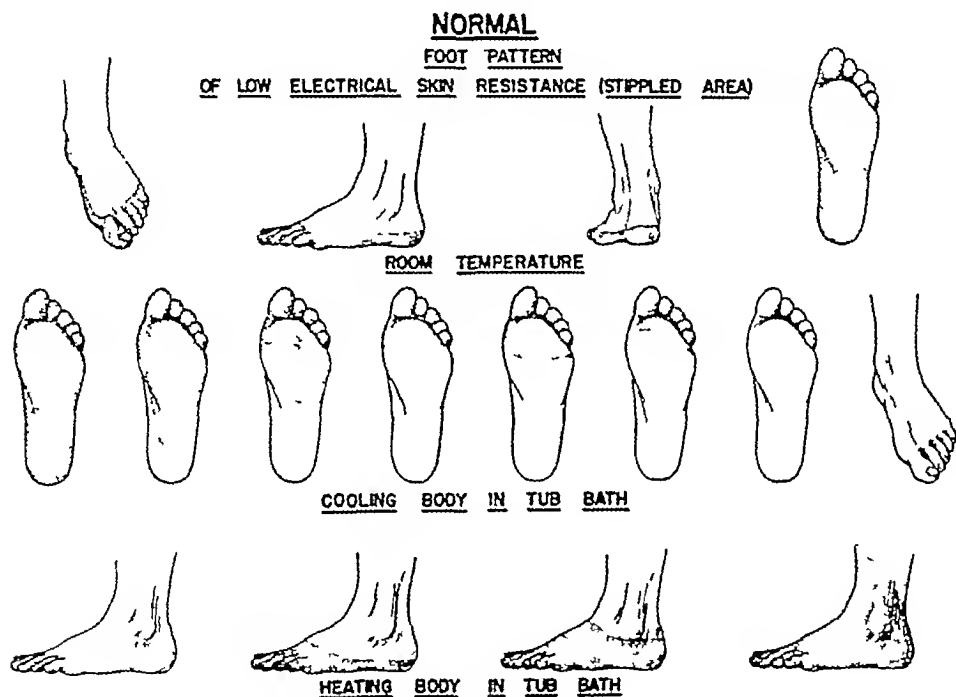


FIG 4

of the finger and thumb to the first joint. During sleep one half hour later this area had become constricted until it included only the tips of the fingers and thumb. Ten babies gave similar records.

**B Feet** The top row in Fig 4 shows the foot pattern of low electrical skin resistance found most frequently at room temperature in normals. It includes the entire plantar surface and a narrow band, about 1 inch in width, which runs around the foot laterally and up over the dorsal surface to include part or all of the dorsal surface of the toes. Less frequently the area includes only the plantar surface and none of the lateral or dorsal areas.

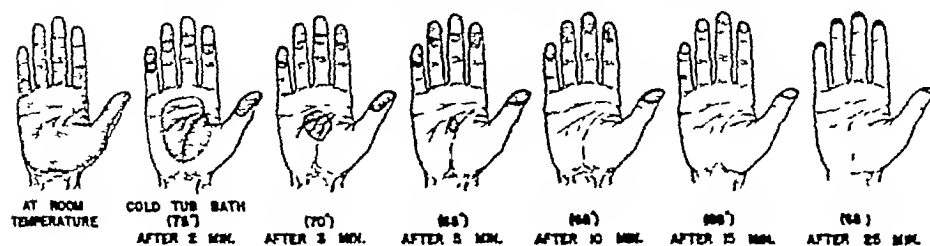
*Effect of temperature changes on foot patterns* In most individuals cooling

the pattern of low resistance of this subject included all of the palmar surface and none of the dorsal surface. After 2 minutes in a bath at 75°F the area of low resistance on the palmar surface became constricted and divided

### TEMPERATURE EXPERIMENT

BODY EXCEPT FOR HANDS IMMersed IN WATER BATH

COOLING



### HEATING

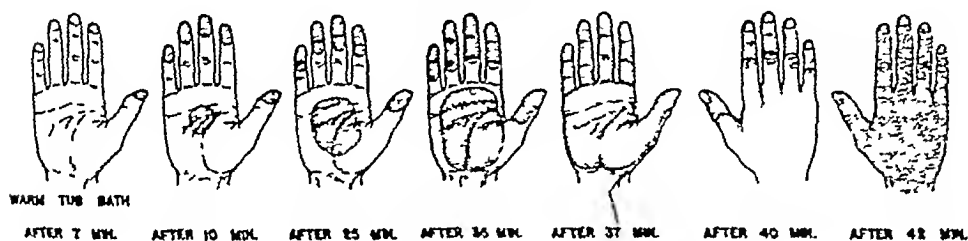


FIG 2

into small areas, one on the tip of each finger and thumb, and another, larger one, in the center of the palm. Progressively, with time and with the lowering of the temperature of the bath, these areas grew smaller until finally after 25 minutes' immersion when the bath had cooled to 68°F, they included only the tips of the fingers and thumbs. At this time hot water was run into the tub gradually.

After 7 minutes the areas on the tips of the fingers had enlarged slightly. After 10 minutes the palm of the hand again showed a small area of low resistance and the areas on the tips of the fingers had become larger. During the next 20-25 minutes the finger, thumb, and palmar areas gradually increased in size until they became consolidated again into a large area which cov-

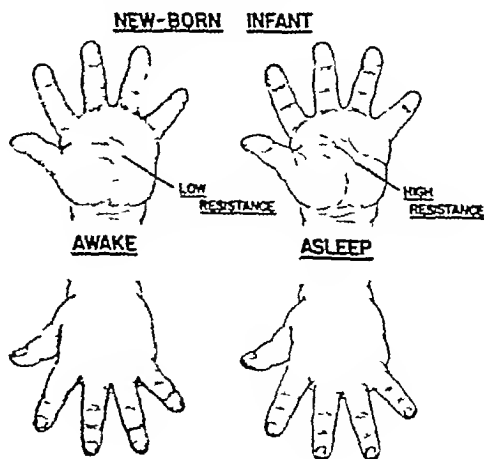


FIG 3



in most individuals, primates as well as human beings, is hairless, is particularly suggestive of a relationship between these two phenomena

*Relation of patterns of low electrical skin resistance to sympathetic nervous system* In observations made on over 50 patients with upper thoracic or lumbar sympathectomies we did not find any pattern of low resistance on hand or feet, even after the patient had been well heated in the hot air cabinet. This would indicate then that the areas of low resistance depend at least in large part on sympathetic innervation of the extremities. In agreement with this conclusion is the observation that the hand and foot patterns become enlarged under conditions in which the sympathetic nervous system becomes activated, such for instance as strong muscular effort or emotional excitement, and become constricted under conditions in which the sympathetic nervous system becomes inhibited, such for instance as sleep and inactivity.

*Relationship of low skin resistance to distribution of nerves* Before discussing the possible relationship between the skin resistance and nerve distribution patterns we must recall some of the most important features of the skin resistance patterns. In the first place, they seem to correspond roughly with sections made perpendicularly to the axis of the arms and legs. When the areas become enlarged they proceed up the extremities in glove and sock patterns, when they become smaller they gradually recede to the most distal ends of the axes, the tips of the fingers and toes. Clearly at no stage do the skin resistance patterns coincide with the distribution of any of the peripheral nerves, such for instance as the ulnar or radial nerves to the hands, or the tibial or peroneal nerves to the feet. Likewise the skin resistance patterns do not coincide with the distribution of any of the segmental nerves. They may agree more closely with the distribution of sympathetic nerve patterns from the cerebral cortex since the work of Guterman and List and others has shown that sympathetic functions like the motor or sensory functions of the legs, arms, and head may be represented in the cerebral cortex (3). The skin resistance patterns seem to include the entire hand and foot, not segmental or peripheral divisions.

## DISCUSSION

The results of this study demonstrate that in normal individuals the skin on the hands and feet, just as on the face, shows hitherto entirely unsuspected patterns of low electrical resistance. Sharp lines, less than  $\frac{1}{4}$  inch in width, usually separate the areas of low from the areas of high resistance. In most instances the resistance in the low areas is about one-fourth as high as in the surrounding areas. It is especially noteworthy that these great differences in resistance exist without any visible differences in the skin. In the previously reported experiments it was found that the facial patterns of low electrical skin resistance coincided with cross sections of the face made perpendicular to the somatic axis of the body and head, that is, regarding the mouth and lips to be the most forward end of the axis in man as well as

of the body in a tub bath of 68°F caused the pattern of low electrical skin resistance to shrink until it included only the spaces between the toes, while heating the body caused the pattern to enlarge first to include only the plantar surface, and a narrow band around the foot, then successively slipper and sock shaped areas, and finally the entire leg. The second and third row of feet in Fig 4 show fairly typical changes.

*Relation of patterns of low skin resistance to sweat glands* These observations showed that under normal conditions the areas of low electrical skin resistance on the hands of normal individuals include all of the palmar surface. It will readily be seen that this area agrees closely with the areas of skin having a rich supply of sweat glands, the so-called porous areas which have a different anatomical appearance from the shape of the back of the hand, not only in structure but in color. Since these areas may often show active sweating while the skin on the backs of the hands is dry, differences other than the number of sweat glands probably distinguish the palmar from the dorsal surfaces of the hands. In moving the electrode from the palmar to the dorsal surface the sharp line of change in resistance usually occurs near the mid-line on each finger, where the porous area of the skin ends, though the low resistance area often includes the dorsal surface of the last two phalanges of the fingers and thumb, as was shown in Fig 1A. This pattern is often obtained in normal individuals under ordinary conditions, and is always found during any kind of emotional excitement or tension. It is not known whether the skin on the dorsal surface of the last two phalanges has a richer supply of sweat glands than the skin on the remainder of the dorsum of the hand. There is on the feet also a close correspondence between the most common pattern of low skin resistance and the porous areas of skin. Here also even under ordinary circumstances the pattern may extend up over the two distal phalanges of the toes, or even up over the entire toes, and up over the edge of the foot.

*Relation of patterns of low electrical resistance to distribution of blood vessels* Anatomically there appears to be very little relation between the skin resistance patterns and the distribution of the blood vessels beyond the fact that the palmar surfaces have a richer supply of blood vessels. In 12 individuals we recorded the patterns before and after complete occlusion of both venous and arterial supply to the hands for as long as 15 minutes and found no consistent changes. In some subjects the patterns contracted until the low resistance area included only the tips of the fingers, just as during sleep or in cold temperatures, while in others, the patterns became enlarged to include not only the hands but also part of the arms. The pain from the hands and arms may have contributed to the enlargement of the patterns.

*Relation between patterns of low electrical resistance and hair distribution* On both the hands and feet, the patterns of low electrical skin resistance under standard conditions were similar to the patterns of hair distribution, having the same shape, but being smaller. The low skin resistance area over the dorsal surface of the last two phalanges of the fingers and thumb, which

sleep, and become enlarged in warm temperatures and with exercise or excitement. When the patterns contract, the tips of the fingers and the toes are the last to show a low resistance. When they expand, the patterns envelop all of the dorsal surface of the hands and feet and then move up the arms and legs, showing regular sock and glove patterns.

3 The possible relationship of these areas to the distribution of sweat glands, blood vessels and hair was considered.

4 It was shown that the patterns do not conform to the distribution of any of the peripheral nerves or the sensory dermatomes. It was suggested that they might represent cortical or sub-cortical patterns of the distribution of sympathetic nerves to the extremities.

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animals It appeared that the patterns correspond closely with the axial segments

The patterns on the hands and arms and on the feet and legs seem to represent analagous cross sections along the axis of the arm and leg respectively As the patterns become constricted, they move toward the ends of the axes, that is, to the tips of the fingers and to the ends of the toes, just as the patterns on the face moved toward the mouth As the patterns enlarge, they move up the axes of the arms and legs in more or less circular patterns These results suggest as we pointed out above, that these patterns must depend on some fundamental neurological mechanisms such as are found only in the higher centers of the brain stem or cortex The facial areas would thus be more likely to be associated with such cortical areas than, as was suggested in the previous paper, with the trigeminal nucleus

It is noteworthy at this point to call attention to the similarity in shape between the sock and glove patterns of low electrical resistance and the patterns of anaesthesia or hyperalgesia often found in hysterical patients The finding of the skin resistance patterns indicates that there may be an anatomical basis for the sock and glove patterns of the hysterics The presence of sock and glove patterns of anaesthesia in peripheral neuritis, has already established the existence of such patterns We have not determined whether these patterns found in the vitamin B<sub>1</sub> deficient patients correspond with the skin resistance patterns

The mapping of the areas of low skin resistance may help to throw some light on the mechanisms involved in the production of various pathological conditions of the extremities, such as are seen in Raynaud's disease, in scleroderma, diabetes, etc It may also be of help in the study of the effects of low and high temperatures on the body In preliminary studies on the effects of heat made during prolonged summer heat spells, we found that in many instances the hand and foot patterns became reversed, the areas of low resistance became in a relative sense the areas of high resistance, that is, the skin on the rest of the body had a lower resistance than that on the palmar and plantar surfaces The mapping of the hand, foot and facial patterns may also be useful in the study of acute and chronic emotional disturbances seen in psychiatric patients

### SUMMARY

- 1 Under normal conditions, that is, at ordinary room temperature, etc , the hands and feet, like the face, show sharply defined areas of low electrical skin resistance On the hands these patterns usually include the entire palmar surface up to the line which divides the dorsal and ventral parts of the hand The skin of this area shows a resistance about one-fourth that of the skin of surrounding areas On the feet the areas of low electrical skin resistance usually include the entire plantar surface and a small band along the side of the foot and over the toes

- 2 These areas become constricted in cold temperatures and during

of certain of the more common tests are tabulated for each animal in Table 2, which illustrates the constancy or variability of the response

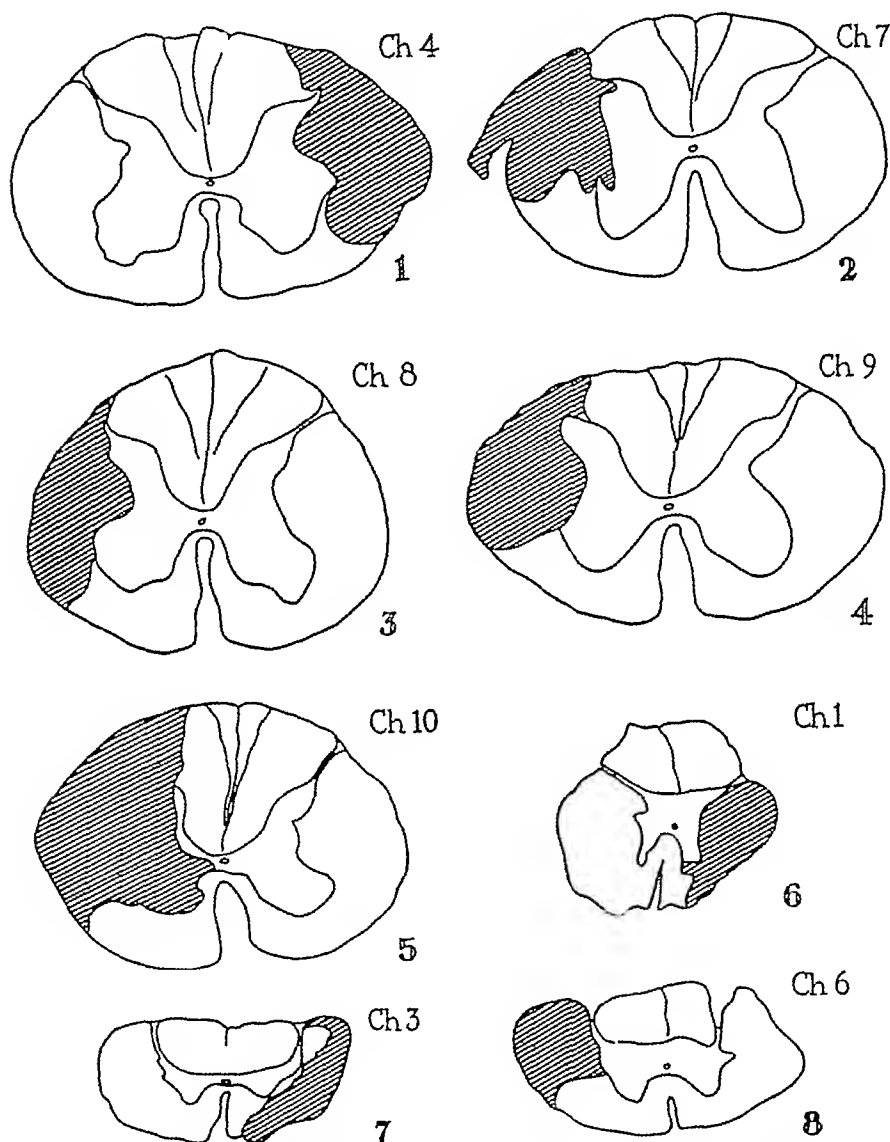


FIG 1 Diagrammatic projection drawings showing destruction of the lateral corticospinal and adjacent ascending and descending tracts in the dorso-lateral column of the spinal cord

In both cervical and thoracic animals the lower extremity of the operated side always exhibited a definite hypotonus on passive manipulation of all muscle groups. There was no evidence of clonus noted in either the

# NATURE OF PARESIS FOLLOWING LATERAL CORTICO-SPINAL SECTION IN MONKEYS\*

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A TRANSIENT contralateral spasticity, consisting of hypertonus, principally extensor in the lower extremity and flexor in the upper, with accompanying hyperactive deep reflexes and clonus, was observed by Hines (2) following ablation of Area 4s (the "strip area") in the rhesus monkey. As yet lesions placed in other areas of the precentral cortex or those placed in the pyramids (4) have failed to give this syndrome. Subsequent to sectioning of the pyramids, Tower (4) observed a paresis characterized by hypotonus, hypoactive deep reflexes, and no evidence of clonus. In the present investigation a similar paresis has been found to exist after the interruption of the lateral cortico-spinal tract at cervical or thoracic levels of the spinal cord.

## METHODS

In a series of 9 *Macaca mulatta* monkeys, dorso-lateral chordotomies were performed, in five instances at cervical (C-4) and in four at thoracic (T-9) levels. Exposure was obtained by a laminectomy and the cord incision was made from the postero-lateral sulcus downward and lateralward to the origin of the dentate ligament.

The results of neurological examinations previous to and at routine intervals after the operation were recorded. ‡ The pre- and post-operative behavior of the monkeys in their cages was also noted. With the exception of one case, still under observation, the animals were sacrificed within four to nine weeks. Sections through the lesion were prepared with the Weil stain and those above and below the lesion, by the Marchi technique. Projection drawings of the lesions are shown in Fig. 1. It should be noted that the lesions involved other tracts of the dorso-lateral column in addition to the lateral cortico-spinal tract.

## RESULTS

In examining the extremity or extremities of the operated side below the level of the lesion, results were recorded in degree of response compared with the response obtained from the corresponding contralateral extremity subjected to the same test. As the extremities of the opposite side presented no apparent changes after operation, this served as a control in eliminating a source of error that might have been introduced by the manifestation of the occasional stoicism found in the monkey.

The predominant response to each test applied is presented in Table 1 which demonstrates the changes that have occurred on the homolateral side subsequent to severance of the lateral corticospinal tract. The results

\* Aided by a grant from the Rockefeller Foundation.

† Medical Fellow of the National Research Council.

‡ Results of post-operative alterations in temperature regulation in these monkeys have been reported by Beaton and Leiminger (1).

The typical response to the placing of an object in contact with the plantar surface of the foot of the normal extremity of the monkey is a strong grasping of the object. The occasional atypical response to such a test is withdrawal of the foot in attempt to avoid the object. The hind limb suffering the paresis failed to show any response whatsoever in all animals except Ch-4 and Ch-8 in which a weak attempt to grasp was noted.

As has been reported by Hines (3), the usual response to plantar stimulation in the normal monkey is an initial extension and then a secondary flexion of the toes at the metatarsophalangeal joints. The affected foot of five monkeys of the present series gave a response much diminished in intensity and frequently not followed by the secondary flexion. Ch-7 gave no response. Ch-3, Ch-6, and Ch-10 showed a moderate response in both hind extremities.

The results of other tests applied, tabulated in Table 1, also deserve consideration. The Achilles tendon reflex of the paretic limb, when elicited in these animals, was hypoactive. The absence of the placing and hopping reactions in the affected limb with the presence of an active cross-placing response further demonstrates the loss of motor control. The abdominal reflex was elicited on the normal side in the animals tested but not on the paretic side. On palpation of the muscles of the calf and thigh, a flabbiness was noted in the limb of the operated side. Atrophy of these muscles was evident after about three weeks, especially noted in the triceps surae group.

The paresis occurring in the forelimb of the animals recovering from cervical tractotomy tended to be more obscure. Passive manipulation showed no indication of the loss of tone in monkeys Ch-7, Ch-8, and Ch-9. However, the paretic forelimb of Ch-4 and Ch-10 exhibited a decreased resistance in both the flexor and extensor group of muscles. The deep reflexes of these affected limbs varied in response from complete absence as in Ch-8 and Ch-10 to no change as in Ch-4 and Ch-9, with Ch-7 demonstrating the intermediate or hyporeflexia stage. Grasping with the paretic hand was very weak and inconsistent in all these animals and Ch-10 gave no response. Palmar stimulation resulted in little response.

In both the cervical and thoracic animals, tests for postural rigidity with the animal hanging in the hammock showed no increase in the tone of the paretic extremities. While hanging, the normal extremities were constantly being flexed. The paretic extremities, however, hung flaccidly extended and were never flexed.

Voluntary control of the movements of the arm at the shoulder suffered only a moderate loss. The loss was more evident at the elbow and was rather complete for digital movements. Gradual improvement was noted in the use of the arm, but digital paresis was permanent. The deficit of motor control was more apparent in the affected leg whose activity was limited to that of a strut in walking. No recovery or compensation was observed for the loss of digital control in the toes. The little tone that was noted some weeks after operation seemed to appear first in the abductors and flexors of

normal or affected limb The usual response to tapping the patellar tendon on the side of operation was a slow to and fro swing of the lower leg in comparison with the quick jerk characteristic of the opposite or normal leg In monkey Ch-4 this pendulous response was replaced by a mere diminution of the response obtained The knee jerks of Ch-10 were of similar activity

Table 1

Test	Lower Extremities	
	Normal	Paretic
Passive manipulation	normal tone	hypotonus
Clonus	absent	absent
Pin prick	normal response	normal response
Patellar reflex	active	pendulous
Achilles reflex	active	hypoactive
Plantar stimulation	active	little response
Grasp	strong	absent
Placing reaction	present	absent
Hopping reaction	present	absent
Postural rigidity	absent	absent
Palpation of muscles	firm	flabby
Atrophy	absent	present
<i>Abdomen</i>		
Abdominal reflex	active	absent
<i>Upper Extremities</i>		
Passive manipulation	normal tone	slight hypotonus
Clonus	absent	absent
Pin prick	normal response	normal response
Biceps reflex	active	hypoactive
Triceps reflex	active	hypoactive
Palmar stimulation	normal	little response
Postural rigidity	absent	absent
Grasp	strong	diminished to absent

on both sides The contraction of the adductors of the opposite thigh was noted to occur frequently in all monkeys when the patellar tendon of the affected limb was struck, but the reverse was not demonstrable

Table 2

		Ch-1	Ch-3	Ch-4	Ch-6	Ch-7	Ch-8	Ch-9	Ch-10	Ch-11
Leg	Muscle tone	<nor	<nor	<nor	<nor	<nor	<nor	<nor	<nor	<nor
	Clonus	abs	abs	abs	abs	abs	abs	abs	abs	abs
	Knee jerk	pend	pend	<nor	pend	pend	pend	pend	nor	pend
	Grasp	abs	abs	<nor	abs	abs	<nor	abs	abs	abs
	Plantar stimulation	<nor	nor	<nor	nor	abs	<nor	<nor	nor	<nor
Arm	Muscle tone			<nor		nor	nor	nor	<nor	
	Deep reflexes			nor		<nor	abs	nor	abs	
	Grasp			<nor		<nor	<nor	<nor	abs	

nor = normal  
 <nor = less than normal  
 abs = absent  
 pend = pendulous





the thigh and flexors of the leg This tone was never sufficient, however, to obscure the definite hypotonus that existed in comparison with the tone of the normal leg

### DISCUSSION

It is evident from these observations that interruption of the lateral cortico-spinal tract in the spinal cord of the monkey gives rise to a paresis in the affected limbs This paresis, which is more apparent in the lower than in the upper extremity, is characterized by a poverty of movement of the entire limb, and also by an apparent lack of voluntary control of movement The paresis is less in the proximal muscle groups and more extreme in the distal, where it approaches a state of paralysis of all digital motion There exists in these paretic limbs a diminished resistance to passive manipulation. This loss of tone is likewise indicated by the flabbiness of the muscles on palpation In addition to this hypotonicity, the hypoactive or pendulous state of the tendon reflexes and the failure of clonus to develop, all definitely indicate the absence of spasticity after section of the lateral corticospinal tract

The present results confirm those of Tower (4) who in 1940 reported that section of the pyramid in the monkey gave rise to a "hypotonic paresis" of the contralateral limbs This term adequately describes the state found to exist in the present experiments after section of the cortico-spinal fibers within the dorso-lateral column at cervical or thoracic levels It is generally believed that there are fibers from higher levels of the brain descending through each half of the spinal cord to the anterior horn cells and exerting an inhibitory influence upon their activity Release from this inhibitory influence is said to result in a syndrome of spasticity characterized by muscular hypertonus, hyperactive reflexes, and clonus If such fibers are present in the spinal cord of the monkey, the results of the experiments reported here indicate that they do not lie within the lateral cortico-spinal tract

### SUMMARY

Interruption of the lateral cortico-spinal tract in the spinal cord of the monkey results in a paresis that is more prominent in the lower than in the upper extremity, and that is more pronounced in the distal than in the proximal muscle groups

This paresis is characterized by hypotonicity, hypoactive reflexes, and absence of clonus, indicating that no descending inhibitory pathway whose interruption results in spasticity is present in the lateral cortico-spinal tract of the monkey

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1 (a) a pseudo-cholinesterase from dog pancreas, prepared according to the method outlined by Mendel and Mundell (2),  $Q_{Ach}$  500,000–700,000, (b) a pseudo-cholinesterase from horse serum, prepared according to a method as yet unpublished,  $Q_{Ach}$  40,000–70,000, 2 a true cholinesterase prepared from the electric organ of *Electrophorus electricus*. A small amount of this material was made available to us through Dr C H Best by Dr D Nachmansohn. The material was purified before using it in our experiments,  $Q_{Ach} > 40,000$ .

### I *Effect of cholinesterase injections on injected acetylcholine*

Acetylcholine when injected into rats brings about an excessive secretion of saliva, intensive lacrimation and the appearance of a material in the tears which greatly resembles blood. This material, an excretory product of the Harderian gland, has been identified spectroscopically as a mixture of protoporphyrin and coproporphyrin. Freud, according to Selye (4), described the shedding of so-called bloody tears after acetylcholine administration. Tashiro and his co-workers (5) proposed that the excretion, to which they applied the name "chromodacryorrhea" be used as a biological assay for acetylcholine. In uneserminized rats they detected the excretion after intraperitoneal injection of 2000  $\gamma$  acetylcholine or after intravenous injection of 10–15  $\gamma$  acetylcholine per 100 g body weight. When administered intravenously to an eserinized rat, however, as little as 0.2  $\gamma$  acetylcholine per 100 g body weight could produce this effect. In our experiments with uneserinized young rats, the reddish tinge characterizing this excretion could always be detected with filter paper within 5 minutes after the subcutaneous injection of as little as 250  $\gamma$  acetylcholine per 100 g body weight.

In order to investigate the effect of injected cholinesterase preparations on the porphyrin excretion resulting from injected acetylcholine the following experiments were carried out: (i) a female rat, weighing 50 g, received 1 mg acetylcholine chloride subcutaneously. Within  $\frac{1}{2}$  minute, porphyrin excretion was evident, the pupils were constricted and excessive salivation was apparent. (ii) a female rat, weighing 50 g, received 1 ml of an aqueous solution of 36.7 units of dog pancreas pseudo-cholinesterase intravenously. Two minutes later 1 mg acetylcholine chloride was given subcutaneously. There was no abnormal salivation, no lacrimation nor evidence of porphyrin excretion, the pupils seemed larger and no light reac-

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releases one molecule of carbon dioxide from the bicarbonate. Therefore, the quotient, volume of  $CO_2$  (in  $\mu$ l)

$$\frac{[\text{time (in hrs)}] \times [\text{dry wt. of enzyme preparation (in mg)}]}{Q_{Ach}}$$

which will be denoted by the symbol  $Q_{Ach}$ , is a measure of the purity of the enzyme preparation.

The amount of enzyme capable of causing the evolution of 1 ml  $CO_2$  per minute at a substrate concentration of 1 per cent acetylcholine for the pseudo-cholinesterase and 10 mg per cent acetylcholine for the true cholinesterase is defined as one unit of cholinesterase.

† Although the activity of the pseudo-cholinesterase is only slight at the low acetylcholine concentrations which probably occur under normal physiological conditions, we have compensated for its small activity at these levels by building up an excessive concentration of this enzyme in the experimental animals.

# REMOVAL OF ACETYLCHOLINE BY CHOLINESTERASE INJECTIONS AND THE EFFECT THEREOF ON NERVE IMPULSE TRANSMISSION

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ALTHOUGH experimental results published during the past twenty years have presented strong indications that acetylcholine is produced in the course of the transmission of nerve impulses from postganglionic cholinergic nerves, from preganglionic endings in the autonomic nervous system, at voluntary motor nerve endings and possibly at central synaptic junctions, the controversy whether acetylcholine is essential to the process of transmission has not as yet been definitely settled

We have attempted to re-investigate this question by utilizing highly purified cholinesterase preparations which would eliminate acetylcholine and thus afford a means of ascertaining its significance. If acetylcholine is essential for synaptic and postganglionic transmission in the parasympathetic nervous system, a reflex which is dependent on the integrity of this system should be abolished if acetylcholine were removed as quickly as it is produced. This, essentially, was the hypothesis tested in the experiments to be reported.

Before attempting to investigate the effect of the removal of acetylcholine on nerve impulse transmission, it was necessary to ascertain whether the cholinesterase preparations to be utilized as an instrument in this investigation could act on acetylcholine injected into the animal body. The present report is therefore divided into two parts: (1) the effect of cholinesterase injections on injected acetylcholine, (2) the effect of acetylcholine removal on nerve impulse transmission. This latter effect was judged with specific relation to one reflex arc.

## *Cholinesterase preparations used*

It has been demonstrated (3) that two types of cholinesterase exist in the animal body: (a) a specific enzyme which hydrolyses only certain esters of choline, exerting its maximum activity at acetylcholine concentrations as low as 3 mg per cent, and displaying increasing inhibition at increased substrate concentrations, (b) a non-specific enzyme, pseudo-cholinesterase, which is capable of hydrolysing both choline and non-choline esters, exhibiting its optimum activity at acetylcholine concentrations above 300 mg per cent—concentrations which are probably above physiological range. Both types of cholinesterase were used.\*†

\* The activity of the enzyme preparation was measured manometrically by Warburg's method at 37.5°C. in a 0.025 M NaHCO<sub>3</sub> solution saturated with 5 per cent carbon dioxide (pH 7.4). Each molecule of acid liberated from the acetylcholine during hydrolysis,

Table 1

Time after injection of Ch-E	Cholinesterase, units/ml blood at 1 per cent acetylcholine	Amt of Ach per 100 g body wt given subcutaneously	Reaction
27 hrs	4 3	1 mg	no salivation, lacrimina
72 hrs	1 92	1 mg	porphyrin excretion slight salivation, but no rin excretion
120 hrs	0 46	1 mg	salivation after $\frac{1}{2}$ min , porphyrin excretion
144 hrs	0 30	1 mg	salivation after $\frac{1}{2}$ min , l tion, but no porphyrin e:
164 hrs	0 17	1 mg	salivation after $\frac{1}{2}$ min , e: porphyrin excretion
188 hrs	0 09	1 mg	salivation and porphyrin tion before $\frac{1}{2}$ min
236 hrs	0 03		
288 hrs	0 02		
336 hrs	0 008 (normal level)		

## II Effect of removal of acetylcholine on nerve impulse transmissio

Since it was evident that the cholinesterase preparations were able on injected acetylcholine within the animal body, it seemed po through the injection of these preparations, to decide whether the re of acetylcholine, physiologically produced, would have any effect on impulse transmission

Up to the present time the majority of the experiments designed vestigate synaptic transmission have been of an electrical nature l experiments, light, the natural stimulus for the reflex investigated w: employed The direct light reflex was brought into action and pupil sizes : as an indicator of the experimental results Engelhart (1) demonstrat: presence of acetylcholine in the iris, ciliary body and aqueous humor eserinizd rabbit's eye, which had previously been exposed to light l could detect no acetylcholine in an eye which had not been subject illumination

### METHOD

Young albino rats, weighing 45-65 g, were used The light source employed stimulus was maintained at a constant distance of 41 inches from the eye, the illum being supplied by a 100W-120V bulb The light intensity reaching the eye thro: aperture in the screen was of the order of 2.5-3 foot candles, as measured with a ph cell An Argus model camera was mounted on an immovable base, to which an : screen was attached at a constant distance from the lens This screen was provided central opening through which the eye would show when the animal was placed c behind it

The results were recorded photographically on infra-red film, the pictures in bot and dark being taken with photoflash bulbs The duration of the flash (1/50 sec) a no time for constriction of the pupil since its outline proved to be sharp in photo taken in the dark

The experimental animals were not anaesthetized since pupil size deviates

tion seemed evident (iii) a female rat, weighing 50 g, received an intravenous injection of 1 ml of an aqueous solution of the same dog pancreas pseudo-cholinesterase preparation, previously inactivated by heat. Two minutes later a subcutaneous injection of 1 mg acetylcholine chloride was given. Within 5 minutes there was excessive salivation and porphyrin excretion, and the pupils were constricted.

These experiments which were repeated on different rats, with the same results, demonstrate that the porphyrin excretion normally following an

*Rate of Disappearance of Horse Serum Pseudo-Cholinesterase  
from the Blood*

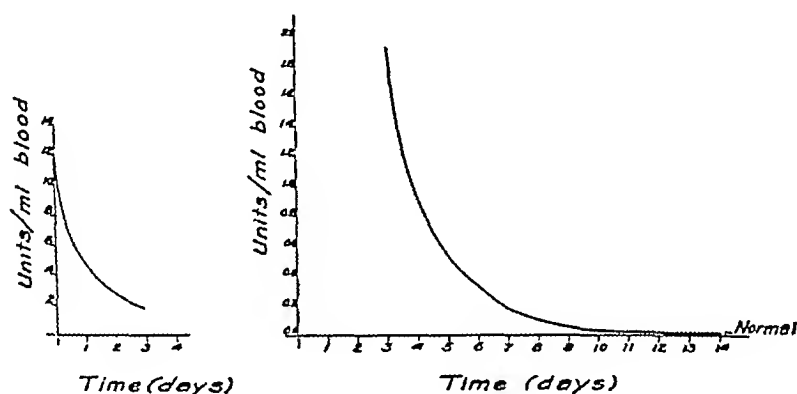


FIG 1

injection of acetylcholine is prevented by an injection of pancreas pseudo-cholinesterase. It is evident that the absence of the porphyrin excretion is due to the enzyme action, since the inactivated preparation is unable to abolish this effect of acetylcholine. Changes in pupil size noted in these experiments were examined subsequently under different conditions and the results will be taken up later in this paper.

Porphyrin excretion could similarly be prevented by the injection of pseudo-cholinesterase from horse serum. Unlike the pseudo-cholinesterase from dog pancreas, which disappears almost completely from the circulation within one hour, the enzyme from horse serum leaves the blood stream very slowly (Fig 1). The effects of a single esterase injection could therefore be followed at different blood-esterase levels in the same animal for a considerable length of time.

Four sets of experiments were carried out with different preparations of pseudo-cholinesterase from horse serum. Table 1 shows the data from one of these experiments. It may be seen that an injection of 60 units of horse serum pseudo-cholinesterase (QAch 66,000) could protect the animal for 164 hours against the chromodacryorrhetic effect of acetylcholine.

*Pupil Sizes After Injection of Pseudo-Cholinesterase  
from Horse Serum*

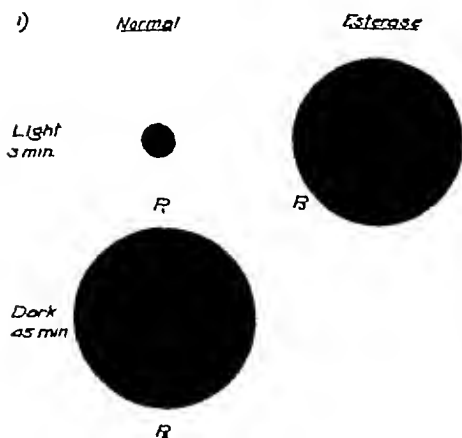


FIG 3  $P_2$  was taken 35 minutes after the injection of the enzyme preparation. All diagrams in this figure are 17 times the original pupil sizes.

Figure 5 represents diagrammatically the pupil sizes of a rat before and after injection of 1 ml of a solution of 60 units of horse serum pseudo-cholinesterase.

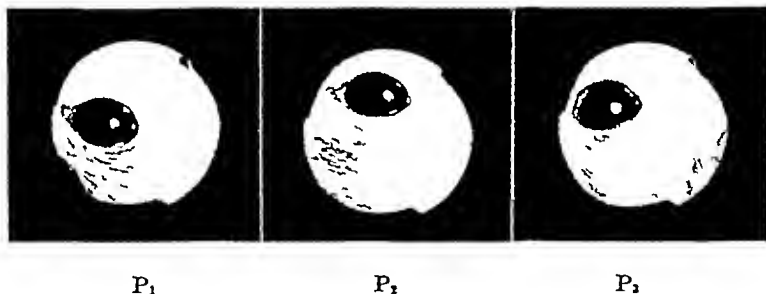


FIG 4 Picture  $P_1$  represents pupil size in the normal rat after an exposure to light of 3 minutes, picture  $P_2$  represents pupil size in the same animal after an exposure to the dark of 45 minutes, picture  $P_3$  represents the pupil size of the same rat after exposure of 3 minutes to light, following injection of 33 units of horse serum pseudo-cholinesterase.

Figure 6 shows the effect of 1 ml of Ringer's solution and of 1 ml of the esterase solution previously inactivated by heat, on the pupil size of another rat.

The latter figure demonstrates that the inactivated preparation causes no further effect on pupil size than the slight dilatation produced by the Ringer's solution. Any inhibition of the constrictor mechanism observed in Fig 5 must therefore be due to the action of the active enzyme.

Figure 7 presents diagrammatically the pupil sizes found before and after the intravenous injection of 5.4 units of true cholinesterase obtained from the electric organ of *Electrophorus electricus*. Since only a limited quantity

anaesthesia and variations in cell permeability under such conditions might also affect the action of injected cholinesterase. In unanaesthetized animals, emotional and psychical changes might give rise to variations in pupil size, but control runs on rats' pupils exposed to light showed that under the experimental conditions marked constriction was always obtained, though its extent varied from one animal to another.

The eye was exposed to the light source for 3 minutes, in order to obtain maximum constriction. A stronger stimulus could have achieved this effect in a shorter time but the animal might then have closed its eye or the unaccustomed light intensity might have served as a fright stimulus. In almost all experiments pupil sizes after the injection of inactivated preparations were recorded to prove that no material capable of causing dilatation was present in the solution. As a basis for comparing the results after injection of inactivated esterase with those obtained in situations involving similar psychical phenomena, pupil sizes after injection of Ringer's solution were recorded in some cases. All solutions used in these experiments were injected into a caudal vein.

## RESULTS

Figure 2 gives a diagrammatic representation of the pupil sizes obtained from a rat before and after an intravenous injection of 0.9 ml. of an aqueous

*Pupil Sizes After Injection of Pseudo Cholinesterase  
from Dog Pancreas*

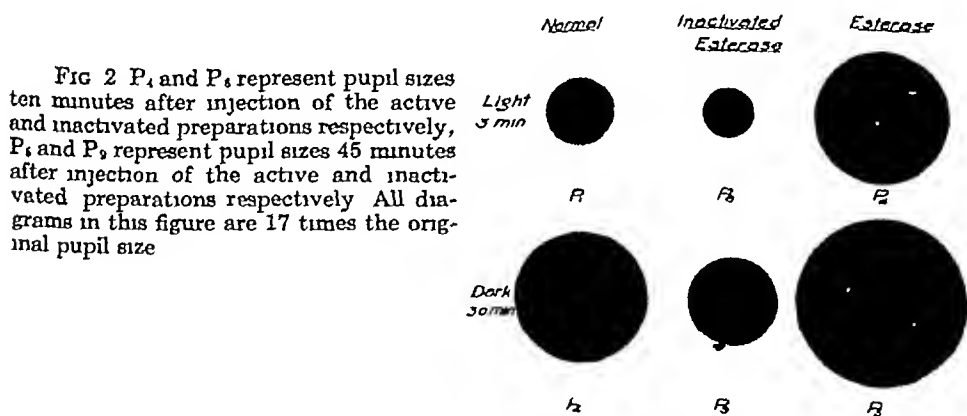


FIG. 2. P<sub>1</sub> and P<sub>2</sub> represent pupil sizes ten minutes after injection of the active and inactivated preparations respectively, P<sub>4</sub> and P<sub>5</sub> represent pupil sizes 45 minutes after injection of the active and inactivated preparations respectively. All diagrams in this figure are 17 times the original pupil size.

solution of 37 units of dog pancreas pseudo-cholinesterase and of 0.9 ml. of the same preparation, previously inactivated by heating for 30 min. at 60°C. It will be seen from this figure that after the injection of the cholinesterase the pupil size in the light (see P<sub>4</sub>) approaches the pupil size recorded on the dark under normal conditions (see P<sub>2</sub>). The pupil sizes after injection of the inactivated esterase seem to indicate the presence of some parasympatheticomimetic substances in the preparation. The experiments with active esterase, however, bear out that the enzyme is able to inhibit the normal light reflex in spite of the action of these constrictor substances. Figure 3 shows diagrammatically the comparative size of the pupil of a male rat before and after the injection of 33 units of horse serum pseudo-cholinesterase. The pupil sizes indicate that the pseudo-cholinesterase from horse serum is also capable of preventing the light reflex almost completely. The actual photographs taken in this experiment are shown in Fig. 4.



## DISCUSSION

Four points exist in the pathway of the direct light reflex where the transmission may be affected by the injected cholinesterase (i) the synapses in the retina, (ii) the central synapses in the oculomotor nuclei, (iii) the synapses in the ciliary ganglion, and (iv) the endings of the short ciliary nerves on the sphincter pupillae

Although it is impossible to decide from the experimental set-up at which point or points the esterase is effective, and although our experiments do not disclose the mechanism by which acetylcholine exerts its effect in the process of transmission, they do provide definite evidence that acetylcholine is essential for the transmission of the impulse to the sphincter pupillae, since its removal by cholinesterase abolishes the continuity of the impulse pathway. Similar experiments with cholinesterase injections on other effector organs innervated by cholinergic nerves must be undertaken, however, before any general statement regarding the role of acetylcholine in impulse transmission can be made.

## SUMMARY

Purified cholinesterase preparations, injected intravenously, are capable of acting within the animal body, thereby preventing the chromodacryorrhetic effect ordinarily obtained from injected acetylcholine.

It has been possible to prove, through the injection of these enzyme preparations, that acetylcholine plays an essential role in the transmission of nerve impulses to the sphincter pupillae. By measuring pupil diameters under constant experimental conditions, it has been demonstrated that the direct light reflex is partially or totally abolished by the injection of cholinesterase preparations, indicating that the integrity of the reflex depends on the presence of acetylcholine at some point or points in the pathway of the nerve impulse.

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of this material was available, it was not possible to investigate its action more fully, but it is evident from the experimental records that this enzyme is also capable of preventing the normal reaction of the pupil to light

*Pupil Sizes After Injection of Pseudo-Cholinesterase from Horse Serum*

2

*Rat I*

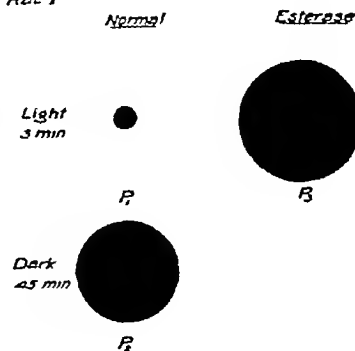


FIG 5  $P_1$  represents pupil size in a rat 15 minutes after the injection of horse serum pseudo-cholinesterase. The diagrams in this figure are 20 times the actual pupil sizes

*Pupil Sizes After Injection of Pseudo-Cholinesterase from Horse Serum*

2)

*Rat II*

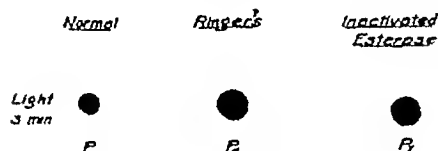


FIG 6  $P_1$  and  $P_2$  represent pupil sizes 28 minutes after the injection of Ringer's solution and inactivated esterase preparation respectively.  $P_1$  and  $P_2$  represent pupil sizes 15 minutes after injection of Ringer's solution and the inactivated enzyme preparation respectively, in the dark. The diagrams in this figure are 17 times the actual pupil sizes

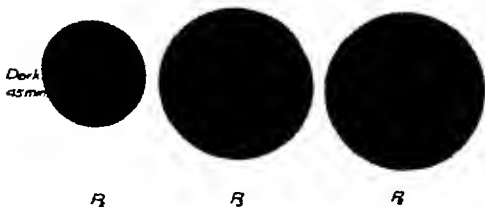
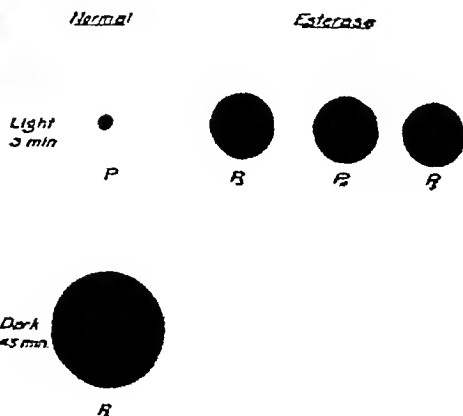


FIG 7  $P_1$ ,  $P_2$  and  $P_3$  represent pupil sizes of the rat at 5 minute intervals, starting 3.5 min. after the injection of 5.4 units of true cholinesterase. The diagrams in this figure are 17 times the actual pupil sizes

*Pupil Sizes After Injection of True Cholinesterase from Electric Eel -*



neck. A condenser coupled amplifier and a commercial cathode ray oscilloscope were used for amplifying and recording potentials of the phrenic nerve. Brief condenser shocks (time constant, 0.1 m sec) were applied to the inspiratory center, or to the descending respiratory pathways in the spinal cord, through bipolar needle electrodes oriented in stereotaxic instruments. A two channel stimulator with independent output circuits was used in these experiments. The stimulator was triggered by a mercury switch activated by a respiratory tambour, and adjusted to deliver the stimuli in mid-inspiration or mid-expiration.

## RESULTS

*Response of phrenic neurons to single shocks applied to the inspiratory center.* A single shock applied to the inspiratory center leads to the discharge

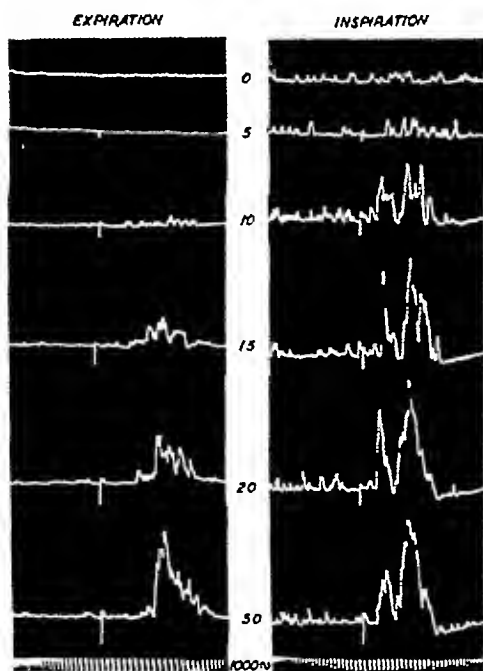


FIG 1 Phrenic nerve response to single shocks of various intensities applied to the inspiratory center during the mid-expiratory and mid-inspiratory phases of the respiratory cycle. Intensities in arbitrary potentiometric units, 50 potentiometric units, approximately 10 peak V.

this intensity produced lesser responses. Thus certain phrenic motor neurons are relatively inaccessible to single volleys of impulses from the inspiratory center in the absence of some facilitative background of respiratory activity.

*Facilitation of phrenic neurons.* We have studied the time course and degree of facilitation of phrenic neurons by quantitating the nerve response to

of a temporally dispersed volley of impulses over the phrenic nerve, lasting some 10 to 20 m sec. As is evident from Fig 1, the latency is brief during inspiration, and averaged about 3 m sec in this and in other experiments, while during expiration, it is prolonged to 6 to 9 m sec. In this experiment, the conduction distance from the stimulating electrodes to the recording electrodes amounted to approximately 100 mm, 55 mm in the medulla and spinal cord, to the 5th cervical root, and 45 mm over the phrenic nerve. Assuming a synaptic delay of about 1.0 msec at the phrenic nucleus (7, 15), the latency during inspiration would suggest that the shortest effective pathway has probably one, and at most two, synaptic breaks. During expiration, on the other hand, the shortest effective pathway must include several neurons. An essentially maximal response was obtained during inspiration, with an arbitrary stimulus intensity of 15,\* while during expiration, stimuli more than three times

\* In this experiment 5 potentiometric units correspond approximately to a peak load voltage of 1V.

# THE BASIS FOR REPETITIVE ACTIVITY IN PHRENIC MOTONEURONS\*

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TRAINS of impulses, conducted over phrenic and dorsal nerves to the diaphragm and intercostal muscles, produce rhythmic inspiratory expansions of the thorax. The frequency of impulses in any given motor unit and the number of motor units active determine the depth of inspiration (1, 3, 4). If the inspiratory center in the medulla oblongata is stimulated with repetitive shocks, these motor units respond, and the frequencies at which they respond, and the numbers which are active, are determined by stimulus frequency and intensity (10). These experiments suggest that a number of descending motor pathways connect the center with the several efferent neurons. They indicate also that the frequency at which any given efferent neuron is caused to fire is related to the average number of impulses reaching it per unit of time, *i.e.* to the number of these pathways activated, and to the frequency at which each transmits impulses. A limiting factor would logically be the instantaneous excitability of the efferent neuron at intervals after it has discharged an impulse. One might well assume a balance between these two factors as the final determinant of frequency.

Typically, phrenic neurons of the cat fire spontaneously at frequencies well below 50 per sec, the majority of those which are active fire from 10 to 30 times per sec at the peak of inspiration. If the above assumptions concerning the determination of discharge frequency are correct, one would predict that excitability is reduced, at least relatively, for periods as long as 100 m sec after the discharge of an impulse. With extreme asphyxial stimulation, however, discharge rates as high as 100 per sec have been observed. Accordingly, one must assume that activation of a sufficient number of spinal respiratory pathways, at a high enough frequency, is capable of re-exciting phrenic neurons even during relative refractoriness. The present study is directed toward the quantitation of changes in excitability exhibited by phrenic motor neurons when they are subjected to volleys of impulses descending from the respiratory center. These experiments bear out the above predictions, and in addition throw further light on the nature of repetitive activity of motor neurons.

## METHODS

Our experiments have been performed on cats anaesthetized with nembutal (30 mg per kg), administered intravenously. The 5th cervical root of the phrenic nerve was isolated, and the other roots sacrificed to obtain the greatest possible length of nerve in the

\* Aided by grants from the Sigma Xi Alumni Research Fund and the Rockefeller Foundation.

tive action on phrenic neurons of the normal inspiratory center discharge. Furthermore, the discharge of these motoneurons, in response to a single shock, is limited to the interval during which facilitation from that shock exists. It would seem that the neurons responding, after all but the briefest latencies, do so only after some degree of facilitation has been developed. Thus relatively long lasting facilitation finds explanation in terms of prolonged delivery of interneuron impulses to the motoneurons over delay pathways or by way of reverberating circuits (8, 14).

*Subnormality in phrenic neurons* If the conditioning shock is increased in intensity, and delivered during inspiration, so as to cause a fair proportion

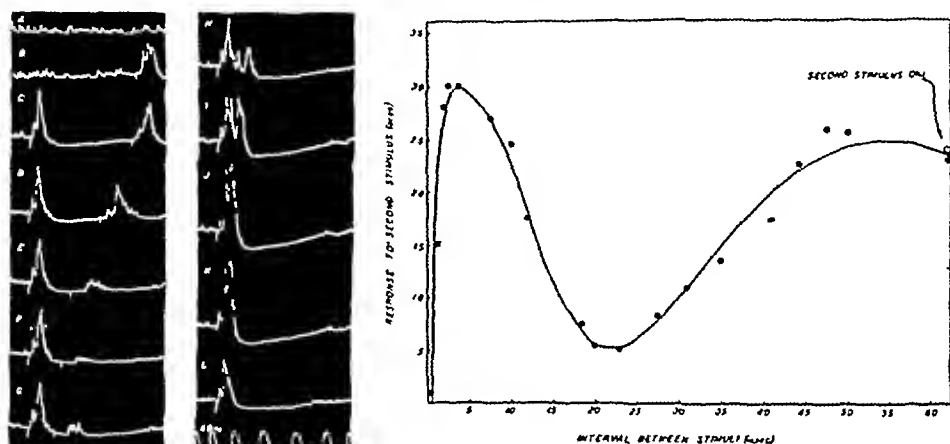


FIG 3 Phrenic nerve response to a testing shock of moderate intensity (3V) following a stronger conditioning shock (8V) at various intervals. Shocks applied during mid-inspiration. In the graph the area of the response to the testing shock is plotted as a function of the interval between the shocks.

of the phrenic neurons to respond, and if the testing shock is also increased somewhat in intensity, a different cycle of excitability change occurs. Figure 3 (C to L) illustrates such an experiment, plotted, on the right, in a fashion identical to that of Fig 2. The test response, unconditioned in record B, is progressively reduced (C to F), as the interval between conditioning and testing shocks is reduced. This extinction of response is most marked when the interval is 20 to 25 msec, as shown on the graph. When the interval is further reduced, the testing response increases in magnitude (G to K), and may show definite facilitation when the interval is about 5 msec. With still shorter intervals, the response drops off again. One is tempted to assume by comparing Fig 2 and 3, that two processes, altering excitability in opposite directions, are simultaneously initiated by strong conditioning stimuli. A weak conditioning stimulus, causing few phrenic neurons to respond, accentuates the facilitative process, Fig 2. A strong conditioning stimulus, causing many phrenic neurons to respond, brings into play simul-

a constant testing stimulus, introduced into the inspiratory center at various intervals after a constant conditioning stimulus. Obviously, the height of a response, as temporally dispersed as those shown in Fig 1, is not a very good indication of number of responding neurons, and hence of the average excitability of the segmental population. Area would seem to be a more quantitative measure of neuron activity. Accordingly response areas were measured, by means of a planimeter, on enlarged tracings of the records. Measurement was complicated by the necessity for introducing a variable factor to correct for non-linearity of the sweep. Since the response itself is inherently somewhat variable, routinely three records were taken at each shock interval, and the response areas for the three averaged.

Figure 2 (C to K) shows the response to a threshold testing shock applied

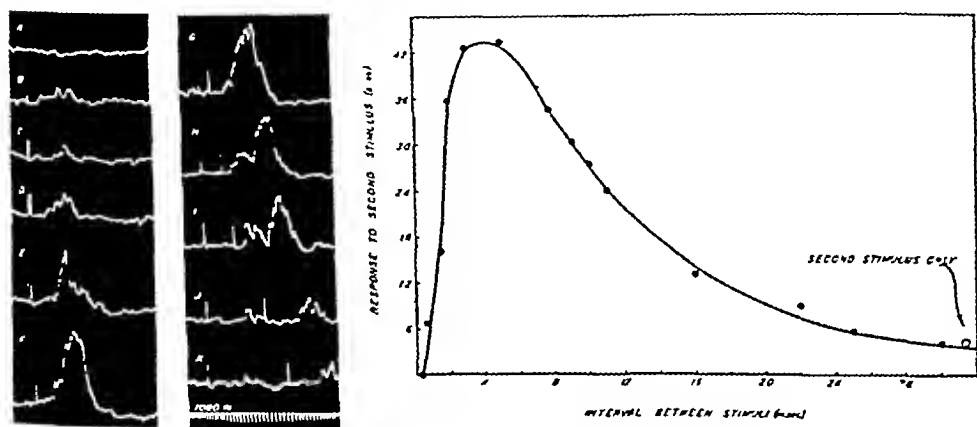


FIG 2 Phrenic nerve response to two equal shocks of threshold intensity applied to the inspiratory center in succession at intervals of 0.5 to 25 msec. Shocks applied during mid-expiration. In the graph the area of the response to the second or testing shock is plotted as a function of the interval between it and the first or conditioning shock.

to the medulla at varying intervals after a conditioning shock of equal threshold intensity. All records were taken during mid-expiration. The average total response area, minus that of the response to the conditioning shock alone, i.e., the area of the conditioned test response, is plotted in the graph on the right, against the interval between the two shocks. Areas are expressed in arbitrary units which are comparable only within a given series of experiments. Facilitation is first evident when the conditioning shock precedes the testing shock by 1.5 msec (E), increases to a maximum when the interval is from 3 to 5 msec (F and G), and disappears after 25 to 30 msec. While facilitation may be readily demonstrated during both inspiration and expiration, it is most apparent in the latter phase, when the inspiratory center is quiescent.

The greater magnitude of phrenic nerve response to a single shock during inspiration than during expiration (Fig 1), finds explanation in the facilitation

given at the left. An intensity of 10\* constituted threshold for the neuron of greater spike potential, 40, for the one of lesser spike potential. Latency diminished from 18 m sec at threshold, to 9 m sec at an intensity of 30, for the neuron of larger potential. For the other neuron latency shortened only slightly at intensities above threshold. The development of a critical degree of facilitation can evidently be hastened considerably in certain instances as a result of increasing the number of descending respiratory pathways active (i.e. by increasing stimulus intensity). In no preparation have we observed a repetitive response from a single shock, no matter how high the intensity. Thus the temporally dispersed character of the discharge, shown in Fig 1, represents for the most part, variation in the time necessary for the several neurons to attain this critical state of facilitation.

In the second group of records titled *facilitation*, two stimuli of approximately half of minimum threshold intensity (one eighth threshold for the neuron of lesser spike potential), were introduced into the respiratory center at varying intervals. When the intervals between conditioning and testing stimuli amounted to 3 to 14 m sec, the neuron of greater spike potential responded (B to E). Within the restricted limits of 3 to 6 m sec, the other neuron, of much higher threshold and lesser spike potential, also responded. The response of this neuron may be observed as a small deflection on the down slope of the spike in record B, which precedes the spike in C.

In the third group of records titled *extinction*, a strong conditioning stimulus, to which both neurons responded (record G), was followed at varying intervals by a moderate test stimulus, to which only the neuron of lower threshold responded (record H). With intervals from 80 to 20 m sec the response to the testing shock was extinguished, while with intervals from 20 to 3 m sec a testing response was again obtained. When the testing stimulus followed the conditioning stimulus at intervals less than 3 m sec, no response was obtained.

Similar experiments during the mid-inspiratory phase of the respiratory cycle were difficult to interpret, for it was impossible, with apparatus available, to introduce a stimulus into the inspiratory center, in fixed relation to a spontaneously fired phrenic neuron impulse. Hence no control could be exerted over the spontaneous fluctuations in excitability of the neuron in question. Results qualitatively similar to those of Fig 4 were obtained in the other seven experiments.

However, as one might anticipate, the intensity of the testing stimulus largely determined the apparent duration of subnormality, and if a testing stimulus of sufficiently high intensity were employed, no phase of extinction of response could be demonstrated in some neurons, beyond a short initial period of unresponsiveness. The use of the single neuron preparation is obviously of limited application in determining the varying magnitude or the time course of an excitability change, since its response is of an all or none

\* In this experiment 10 potentiometric units correspond approximately to a peak load voltage of 3V.

taneously both facilitation and subnormality. Early in recovery from such a strong conditioning stimulus, a more short-lived facilitation might outweigh depression of excitability, but later, a longer lasting depression would become more evident. It is interesting to observe that the spontaneous discharge of impulses into the phrenic nerve (Fig. 3A) is abolished for the duration of the period of subnormality (note especially H to L).

*Facilitation and subnormality in single motor units.* To relate these average

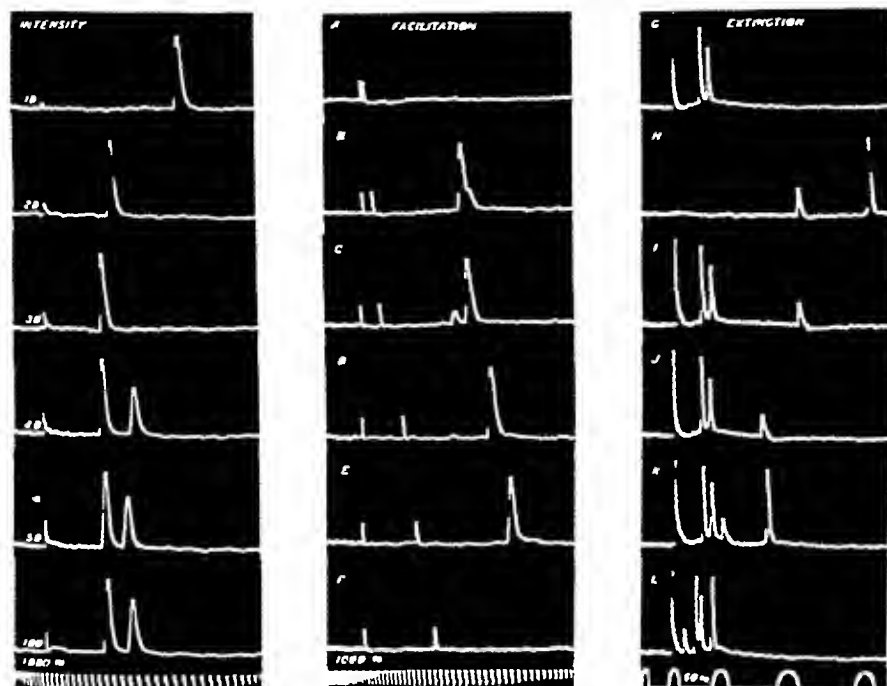


FIG. 4. Response of two phrenic neurons to shocks applied to the inspiratory center during mid-expiration. *Intensity* records, single shocks at various intensities from 10 to 100 arbitrary potentiometric units (approximately 3 to 30V). *Facilitation* records, two equal subthreshold shocks at intervals of 1 to 16 msec. *Extinction* records, conditioning shock of 15V followed at various intervals by a testing shock of 6V. The first deflections in records G and H are the shock artifacts of the conditioning and testing shocks respectively.

changes in excitability of a segmental population of motoneurons to the behavior of the individual units of the group, we have studied some eight single or double phrenic neuron preparations under similar experimental conditions. In Fig. 4 are presented records from one such preparation, in which the responses of two neurons, distinguishable by differences in spike potential, are evident. Gradual deterioration of the end of the fiber accounts for the reduced response of the neuron of lesser potential in the records titled *facilitation*. All records were taken during mid-expiration. In the first series of records, single shocks of progressively increasing intensity were applied to the inspiratory center. Intensities, in arbitrary potentiometric units, are



compares the excitability changes following a strong conditioning stimulus, when measured by testing shocks of moderate intensity (open circles), and when measured by shocks of high intensity (solid circles). The times of the initial peak, the succeeding depression, and the final return to normal excitability are not significantly different in the two instances, although the changes are accentuated by using weaker testing stimuli.

Choice of intensity of the testing shock alters apparent magnitude but not time course of the excitability changes which follow conditioning shocks of fixed intensity, choice of intensity of the conditioning shock obviously alters both magnitude and time course, and as well, the character of these

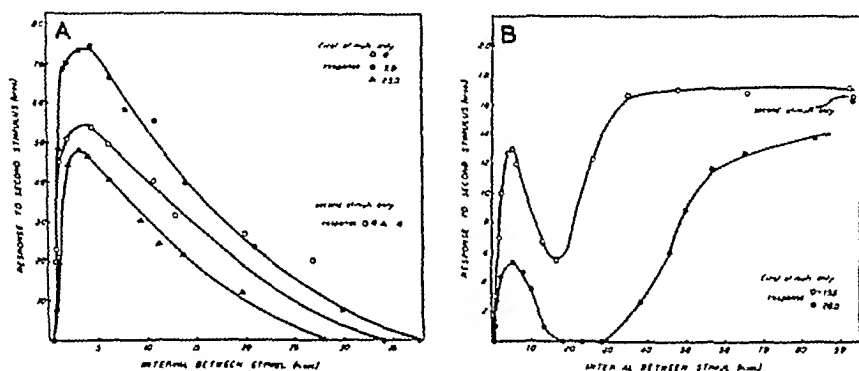


FIG 6A A comparison of the changes in excitability resulting from conditioning shocks of three intensities: subthreshold (open circles), low intensity (solid circles), moderate intensity (triangles). The testing shocks were equal and subthreshold for all. Stimuli were applied to the inspiratory center during mid-expiration. Note that the degree of facilitation at first was increased and then diminished as a result of progressive increase in intensity of the conditioning stimulus.

FIG 6B A comparison of the changes in excitability resulting from strong conditioning shocks (open circles) and essentially maximal conditioning shocks (solid circles). The testing shocks were moderate and of such intensity as to permit quantitation of both increases and decreases in excitability. Stimuli were applied to the inspiratory center during mid-inspiration.

changes. In the experiment shown in Fig. 6A, just subthreshold shocks were used to test excitability following conditioning shocks of three intensities: just subthreshold (open circles), low intensity (solid circles), and moderate intensity (triangles). All stimuli were applied to the inspiratory center during mid-expiration. Marked facilitation follows conditioning shocks of all three intensities, but there is evident a somewhat perplexing variation with intensity in the degree of facilitation. Facilitation increased as the intensity of the conditioning stimulus was raised from below threshold values to slightly above threshold, and then decreased as intensity of the conditioning stimulus was further raised, as may be seen in the lower curve of Fig. 6A. This apparent decrease in facilitation sets in at an intensity of the conditioning stimulus which leads to significant motoneuron discharge, and in general, the reduction is related to the magnitude of that discharge.

character Accordingly experiments were performed, utilizing the entire 5th root of the phrenic nerve, to determine in what way intensity of testing and conditioning stimuli modify these apparent changes in excitability

*Influence of testing shock and conditioning shock intensity on apparent excitability change* Figure 5A is a comparison of the apparent changes in excitability, following a near threshold conditioning stimulus, when they are measured by testing shocks near threshold (open circles), and when they are measured by shocks of moderate intensity\* (solid circles) The stimuli were applied to the inspiratory center during mid-expiration For both curves,

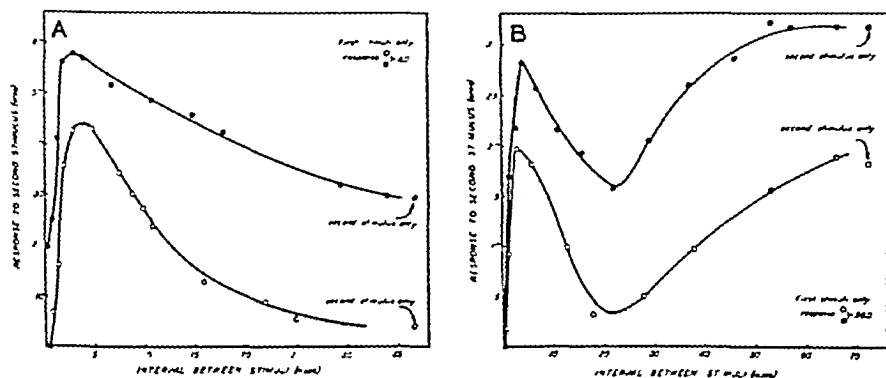


FIG 5A A comparison of the changes in excitability resulting from threshold conditioning shocks when measured by testing shocks near threshold (open circles) and of moderate intensity (solid circles) Stimuli were applied to the inspiratory center during mid-expiration

FIG 5B A comparison of the changes in excitability resulting from strong conditioning shocks when measured by testing shocks of moderate intensity (open circles) and high intensity (solid circles) Stimuli were applied to the inspiratory center during mid-inspiration

the response to the conditioning stimulus in isolation amounted in arbitrary area units to 4 The response to the weaker testing stimulus in isolation was also 4, while to the stronger testing stimulus the response amounted to 30 It is apparent from these curves that the average time course of the facilitation which results from such low intensity conditioning stimuli is not altered by the choice of the intensity of the testing shock, though the relative degree of facilitation is greater when measured with weaker testing shocks Similar conclusions may be drawn concerning the effect of testing shock intensity on the measurement of excitability after strong conditioning stimuli, applied to the inspiratory center during mid-inspiration Figure 5B

\* In this and succeeding experiments we have expressed intensities in relative descriptive terms We have done so because we feel that peak load voltages have little significance in the absence of knowledge of exact distances of the stimulating electrode tips from the excitable structures Slight variations in the position of the electrodes greatly alter threshold stimulus intensity (9), and obviously in our several experiments these variations have been significant Intensities in all our experiments have been within the range of 1 to 30V, for the most part within 1 to 15V

is plotted as the zero reference level. Variations above this reference level indicate facilitation, variations below, indicate subnormality. Subtracting curve B from A, yields C. The actual form of the initial part of curve C, and the extent of the subnormality which it indicates should be considered as having only qualitative significance. For instance, slight displacements of curves A and B which are well within the range of experimental variability, may considerably alter the initial part of curve C. Also it is quite reasonable

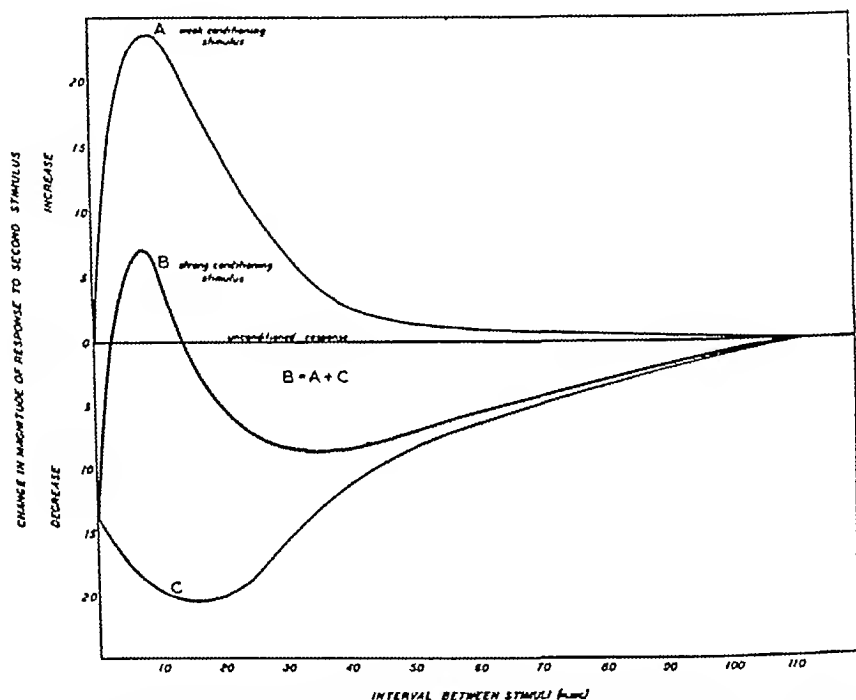


FIG. 8. A graphic analysis of the changes in excitability produced by weak and strong conditioning shocks applied to the inspiratory center. Data obtained from Fig. 7, explanation of the analysis in the text.

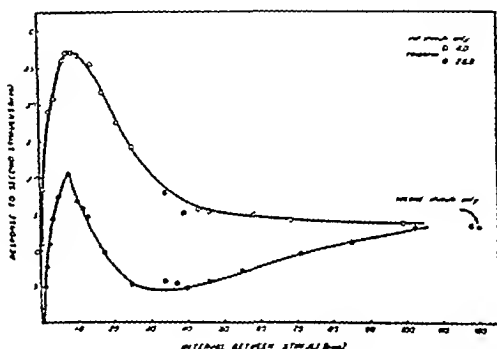
to assume that the actual magnitude of the facilitative process is greater with the stronger conditioning shock than with the weaker. Hence the extent of the depression of excitability is really much greater than that shown by curve C. But such a plot does illustrate that a strong conditioning stimulus initiates two independent processes, one, characterized by increased excitability, the other by depressed excitability. In all of our experiments, the latter change seems correlated with the numbers of responding motoneurons.

*Site of excitability change.* The possible sites at which these changes in excitability might occur, and hence the structures involved are sufficiently few in number to encourage an attempt at their elucidation. Experiments to be presented indicate that facilitation results largely from repetitive activity of inspiratory center neurons, and only to a minor degree from repetitive

The process may be followed further by increasing the intensity of the test stimulus to such a value that both increases and decreases in excitability may be quantitated. Such an experiment is illustrated in Fig. 6B, in which, during mid-inspiration, the excitability changes were followed after strong (open circles), and essentially maximal (solid circles) conditioning shocks. Both degree and duration of subnormality increased when greater numbers of motoneurons were caused to fire by increasing the intensity of the conditioning shock.

In the experiments outlined above we have arbitrarily chosen to demonstrate increases in excitability following weak conditioning shocks by delivering them to the inspiratory center during mid-expiration. To demonstrate depression of excitability, on the other hand, strong conditioning

FIG. 7. A comparison of changes in excitability produced by threshold and by strong conditioning shocks. The testing shocks were moderate and of such intensity as to permit quantitation of both increases and decreases in excitability. Stimuli were applied to the inspiratory center during mid-expiration.



shocks have been applied during mid-inspiration. The reason for this choice lies in the fact that even weak conditioning stimuli, introduced during inspiration, cause the firing of appreciable numbers of phrenic motoneurons. These neurons are apparently already somewhat facilitated by the impulses from an active inspiratory center. Hence even though an initial phase of facilitation may be demonstrated following the weak stimulus, it is succeeded by appreciable subnormality. On the other hand, a weak or even moderate stimulus during expiration may produce marked facilitation, yet, since few motoneurons discharge, no reduced excitability follows. By proper choice of intensities of both conditioning and testing stimuli, however, the same phenomena may be demonstrated in either phase of the respiratory cycle.

*Dual nature of excitability change following strong shocks.* To illustrate further the dual nature of the changes in excitability produced by strong stimuli, two intensities of conditioning stimuli were chosen, one of which produced facilitation alone, the other, facilitation followed by subnormality. The stimuli were applied to the inspiratory center during mid-expiration, and excitability was quantitated by a constant test stimulus. The experimental data are shown in Fig. 7, and the smoothed curves are plotted as A and B, in Fig. 8. In the latter figure, the magnitude of the unconditioned test response

5 m sec (F, G, H) Extinction of the testing response occurred at 8 m sec and for a considerable period thereafter Since latency amounted to about 2 m sec in this experiment for a total conduction distance of 70 mm, the response to a single volley of impulses (I, J) must represent direct activation of some phrenic neurons by endings of tract fibers The facilitated responses (C to G) probably represent the addition of other neurons discharged as a

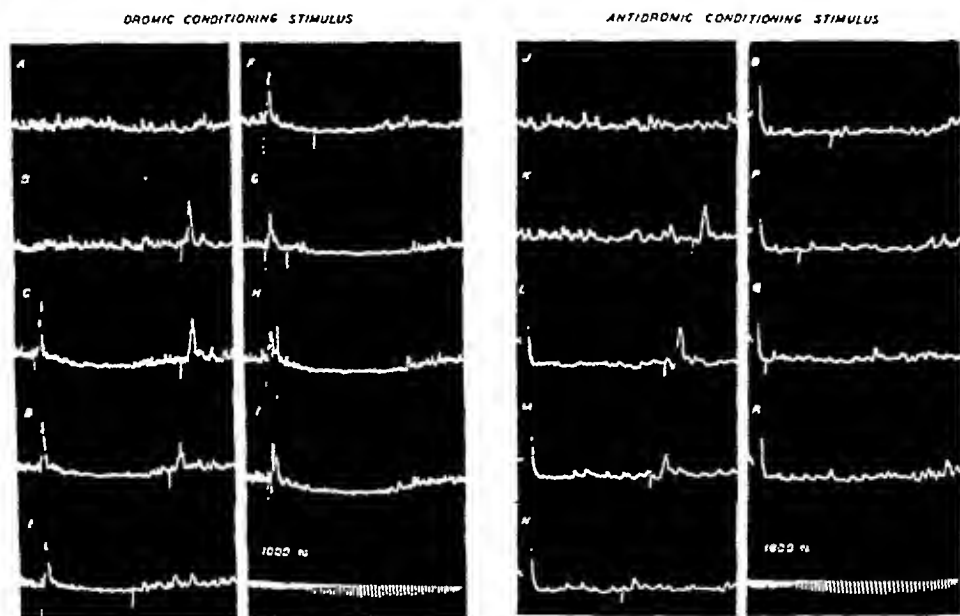


FIG. 10 A comparison of degree and time course of subnormality of phrenic neurons induced by dromic and antidromic excitation. The testing shocks in both instances were applied to the spinal respiratory pathways. In the records of dromic excitation, the conditioning shocks were applied to the spinal pathways through the same electrodes. In the records of antidromic excitation, the conditioning shocks were applied to the phrenic nerve.

result of coincidence of the second tract volley and local internuncial impulses set up by the first volley. For the very brief shock intervals in records C and D, a single relay interneuron is probably involved. For the intervals shown in records E to G, a short chain of interneurons probably continues to deliver impulses for some 6 m sec.

A comparison of the records of Fig. 2 and 9, however, illustrates the fact that facilitation (as indicated by an increase in response area) is less striking and much less persistent following a conditioning shock to the descending respiratory tracts, than when applied to the center. This is emphasized by the fact that significant responses could not be obtained with single stimuli applied to the tracts during expiration, *i.e.* in the absence of a facilitative background of inspiratory activity. We may assign the major part of the facilitation produced by a single conditioning stimulus in the medulla to

spinal interneuron activity. Subnormality, on the other hand, is largely resident in phrenic motoneurons, and to a lesser extent in spinal interneurons and respiratory center neurons. A number of experiments were performed in which the spinal cord was systematically stimulated at the first cervical level with minute needle electrodes oriented to various depths with an adjustable carriage attached to the Horsley-Clarke instrument. A response latency, significantly shorter than that observed on stimulation of the inspiratory center, was the criterion chosen to indicate a descending respiratory tract response.

In initial experiments, exploration of the cord was carried out with single

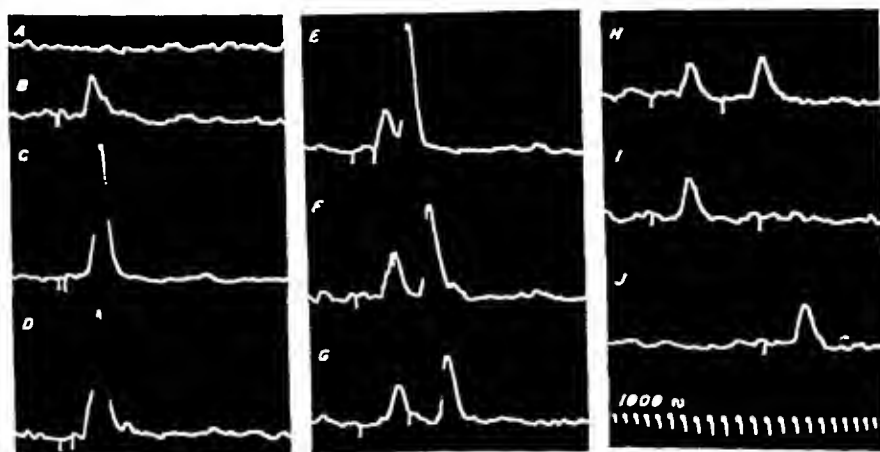


FIG. 9 Phrenic nerve response to two equal shocks of low intensity applied in succession to the descending spinal respiratory pathways at intervals of 0.3 to 8.0 msec. Stimuli applied during mid-inspiration.

shocks applied during mid-expiration. Under these conditions, no responses of short latency were obtained from any part of the cord. However, from the lateral columns there were obtained responses similar to those shown in Fig. 1, but with significantly longer latencies. These responses undoubtedly represent effects of stimulating afferent pathways with relay through the respiratory center. In subsequent experiments, in which shocks were applied during mid-inspiration, responses of brief latency were obtained from the anterior and antero-lateral columns of the cord. These responses differed from those obtained on stimulation of the inspiratory center in that the latency was shorter and the discharge more nearly synchronous. In Fig. 9 are presented records obtained when two equal shocks of low intensity were applied to the anterior columns during mid-inspiration. The response to the conditioning shock alone is shown in record I, to the testing shock alone, in record J. With intervals of 0.3 msec or less, no response was obtained to the testing shock. When the interval amounted to 0.7 to 1.7 msec definite facilitation was observed (C, D, E), which diminished as the interval was prolonged to

spiratory center with repetitive stimuli of various intensities and frequencies (10)

The present investigation throws light on the origin of the synaptic barrage impinging on the final motoneuron, and on the magnitude and time course of subnormality of that neuron once it has been caused to fire. A

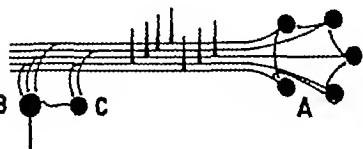


FIG 11 A diagrammatic representation of inspiratory center phrenic neuron relationships. A, inspiratory center, the neurons of which are connected in a reverberating circuit such that a single shock applied to the center leads to a protracted volley of impulses over the descending spinal pathways B, phrenic neurons, receiving some direct respiratory tract collaterals and also terminations of local spinal interneurons, C

Some direct tract fibers impinge on motoneuron B and interneuron C, but in the absence of spontaneous activity in the system, the impulses delivered by the direct collaterals serve only to lower the threshold of the motoneuron to succeeding impulses from center and interneuron. Thus latency is longer in expiration than in inspiration (Fig 1), for in the latter phase, an already facilitated motoneuron responds to the initial direct tract impulses. Spinal interneurons must be relatively few in number for facilitation is brief following a single shock to the cord, lasting only a few m sec (Fig 9)

An exact measurement of the absolute refractory phase for phrenic

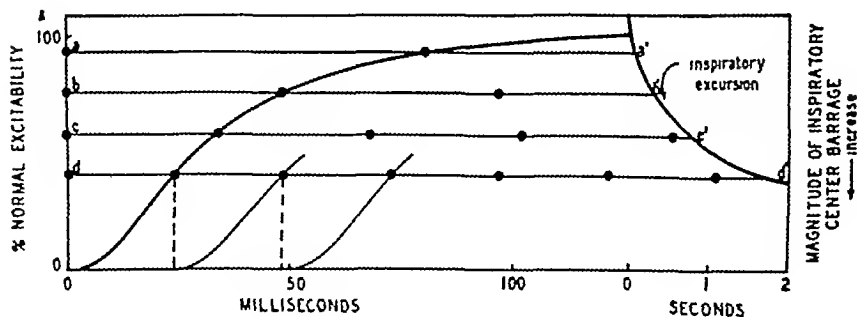


FIG 12 A schematic representation of those factors which play a role in the regulation of frequency of phrenic motoneuron discharge (modified after Adrian and Zotterman, 2). The heavy curve represents recovery of excitability of a phrenic neuron once it has discharged an impulse. The horizontal lines represent the magnitudes of the inspiratory center barrage impinging on that neuron at various times during the developing inspiration. The neuron will again fire an impulse when it recovers to a point where its instantaneous excitability is equal to the maintained level of excitation. The dots on the horizontal lines represent the times at which impulses will be discharged by the neuron at successive intervals during inspiration.

repetitive activity of inspiratory center neurons. These neurons have been shown to be extensively interconnected (10, 12) and are probably arranged in reverberating or reentry circuits.

Subnormality in these experiments did not seem to be essentially different from that observed in previous experiments (*cf* Fig 3), except for its earlier appearance (record 9I). To determine whether this subnormality following discharge of phrenic motoneurons is resident in these neurons or in the surrounding internuncial pool, excitability was tested by a tract shock, following antidromic and tract conditioning shocks. The 4th, 5th and 6th cervical dorsal roots were sectioned ipsilaterally, and the 5th cervical phrenic root used for recording and stimulating. One such experiment is illustrated in Fig 10. On the left (records A to I), a strong tract conditioning shock was followed by a moderate tract testing shock. On the right (records J to R), the same testing shock was applied following an antidromic conditioning shock applied to the phrenic nerve. Due to blocking of the amplifier, a maximal antidromic shock could not be used. All records were made during mid-inspiration.

As may be seen by comparing the two sets of records, essentially the same time course of reduced excitability was observed following trans-synaptic and antidromic excitation of phrenic neurons. Despite the difficulties in interpreting results of antidromic excitation (6), these experiments would seem to point to the phrenic neurons as the site of the major depression of excitability. However, these experiments do not rule out the possibility that respiratory center neurons or local spinal interneurons may also show subnormality as a result of intense activity initiated by a strong conditioning shock.

## DISCUSSION

The repetitive initiation of nerve impulses by a sensory receptor has been explained by Adrian and Zotterman (2) in terms of the time course of recovery of excitability of the receptor and the intensity of the maintained stimulus. For a brief interval after an impulse has been initiated, the threshold of the receptor is infinite, recovery then begins, and the threshold falls gradually toward the resting level. The higher the intensity of the maintained stimulus, the sooner within the recovery period will it exceed the decreasing threshold of the receptor, and the earlier will another impulse be initiated. As was inferred by these investigators, this concept may be applied to repetitive discharge of motor neurons, if one substitutes for the constant stimulus acting on the sensory receptor, a more or less statistically constant barrage of impulses impinging on the motor neuron by way of the many end feet covering its soma and terminal dendrites. The frequency at which impulses are initiated by the motor neuron becomes a function of the time course of recovery of its excitability and the average intensity of this synaptic bombardment. This concept has been applied in explaining the discharge of phrenic motoneurons resulting from stimulation of the in-



center to motoneuron as a result of delay pathways or reentry circuits within the center. Spinal interneuron repetitive activity plays a much less prominent role. Subnormality on the other hand is mainly resident within the phrenic motoneurons.

The repetitive discharge of phrenic neurons which characterizes normal inspiratory activity may be explained in terms of a balance between the degree of excitation of those neurons and their rates of recovery of excitability.

**Acknowledgment** The author is indebted to Mr. John Hervey, of the Johnson Foundation, for the design of the stimulator used in this investigation and to Dr. Joseph C. Hinsey and Captain Eugene F. Du Bois (MC), USNR, for constructive criticism of the manuscript.

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motoneurons has not been possible but it would seem to lie between 1 and 3 m sec. But relative refractoriness, diminishing in magnitude, lasts up to 100 m sec following discharge (Fig 7). For about 1 m sec following a strong shock to the center, no response to a second shock may be obtained. However there continues to be delivered to the motoneuron an increasing barrage of impulses, which more than offsets the depressed excitability after 5 m sec or so. As this barrage diminishes in magnitude, the more prolonged depression of excitability becomes evident, and may completely extinguish the response to the testing shock at about 30 m sec (Fig 8). This depression of excitability is presumed to be resident chiefly in the phrenic motoneurons on the basis of antidromic excitation experiments.

The conditions which obtain in normal respiration are obviously considerably different from those described above. A diagrammatic representation of the factors controlling normal phrenic motoneuron discharge are given in Fig 12. During inspiration, the discharge of impulses from the center progressively increases, paralleling the degree of inspiratory expansion of the thorax ( $a'd'$ ), until the inhibitory inflow from pulmonary afferents and the brainstem inhibitory system (pneumotaxic center) cuts them off sharply,  $d'$  (11, 13). The horizontal lines  $a-a'$ ,  $b-b'$ ,  $c-c'$ ,  $d-d'$  represent magnitudes of this barrage of impulses (*i.e.* total number of impulses per unit time) impinging on a given phrenic neuron at any instant. These levels of excitation may be considered in relation to the curve on the left which represents recovery of excitability of that neuron once it has discharged an impulse. The neuron will again fire an impulse when it recovers to a point where its instantaneous excitability is equal to the maintained level of excitation. Thus the dots on the horizontal lines represent the times at which succeeding impulses will be discharged for each level of excitation during the developing inspiration. Such a plot illustrates the basis for the characteristic slowly augmenting and rapidly decreasing frequency of discharge of phrenic units during the inspiratory cycle (5). The factors underlying the phasic variation in excitatory outflow from the inspiratory center have been treated elsewhere (11, 13).

#### SUMMARY

A single shock, applied to the inspiratory center in the medulla oblongata of the cat, leads to the discharge of impulses over spinal respiratory pathways for periods of 30 m sec or more. If the stimulus is weak and applied during expiration, it will cause few phrenic neurons to respond, but will facilitate those neurons to subsequent shocks if they follow the first at intervals of less than 30 m sec. On the other hand, a strong stimulus produces this same facilitation, but since it causes large numbers of phrenic neurons to respond, it initiates subnormality in those neurons. For an initial period of 20 m sec, the more short-lived facilitation outweighs subnormality, but the latter dominates the picture during the succeeding 100 m sec.

Facilitation largely results from the continued delivery of impulses from

- Epileptiform attacks, 73
- Fibers, long association, 129
- , sensory, accommodation, 337
- , —, autorhythmic mechanism, 337
- FINLEY, K H, See MORISON, R S, 243
- Foot patterns, skin resistance, 417
- Forebrain structures, electrical activity, 243
- FREUND, S, and SHEEHAN, D Experimental investigation of visceral afferent synapses in coeliac ganglia, 263
- Frog, epileptiform attacks, sudden cooling, 73
- Frontal lobes, orbital surface, 349
- GALAMBOS, R, and DAVIS, H The response of single auditory-nerve fibers to acoustic stimulation, 39
- Galvanic skin reflex, 191
- Ganglia, basal, electroencephalogram, 405
- , coeliac, afferent synapses, 263
- GAROL, H W, See BAILEY, P, 121, 129
- GELLHORN, E, and MINATOYA, H The effect of insulin hypoglycemia on conditioned reflexes, 161
- Gland, sweat, responses to stimulation, 191
- GRANT, R, and SKOGLUND, C R Accommodation and autorhythmic mechanism in single sensory fibres, 337
- GURDJIAN, E S, See WEBSTER, J E, 255
- HALSTEAD, W C, See WALKER, A E, 213
- Hand patterns, skin resistance, 417
- HAWKINS, R D, See MENDEL, B, 431
- Hemisection in monkey, 155
- HOFF, H E, See RUBIN, M A, 23
- HUGHES, J, See MCCOUCH, G P, 155
- HUNTER, J, See SOLANDT, D Y, 17
- Hypoglycemia, acetylcholine level, 329
- , insulin, 161
- Hypothalamic lesions and cerebral cortex, 81
- Hypothalamus lesion, electroencephalogram, 405
- Insulin hypoglycemia, 161
- KENNARD, M A Electroencephalogram of decorticate monkeys, 233
- , Effects on EEG of chronic lesions of basal ganglia, thalamus and hypothalamus of monkeys, 405
- , and McCULLOCH, W S Motor response to stimulation of cerebral cortex in absence of areas 4 and 6 (*Macaca mulatta*), 181
- KLINE, R F, See BRITTON, S W, 65
- KUFFLER, S W Specific excitability of the end-plate region in normal and denervated muscle, 99
- LEININGER, C R, See BEATON, 29, 37
- Lesions, cortical, delayed alternation, 173
- LLOYD, D P C Reflex action in relation to pattern and peripheral source of afferent stimulation, 111
- , The interaction of antidromic and orthodromic volleys in a segmental spinal motor nucleus, 143
- , Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat, 298
- , Conduction and synaptic transmission of reflex response to stretch in spinal cats, 317
- Lobe, temporal, monkey, 121
- , temporal, chimpanzee, 121
- Localization, cortical, in rat, 173
- LOTHROP, G H, See MORISON, R S, 243
- Macaca mulatta*, See Monkey
- MCCOUCH, G P, HUGHES, J, and STEWART, W B The monkey (*Macaca mulatta*) after hemisection and subsequent transection of spinal cord, 155
- MCCULLOCH, W S, See BAILEY, P, 121, 129
- , See KENNARD, M A, 181
- MACHADO, A L, See NACHMANSOHN, D, 203, 383, 397
- McKINLEY, W A, See BEATON, L E, 29
- Magnesium, intravenous, electrogram of cat, 23
- MARSHALL, W H, TALBOT, S A, and ADES, H W Cortical response of the anesthetized cat to gross photic and electrical afferent stimulation, 1
- , See WOOLSEY, C N, 287
- Medulla, oxygen tension, 135
- , metabolism, 135
- , salivatory center, 195
- MENDEL, B, and HAWKINS, R D Removal of acetylcholine by cholinesterase injections and the effect thereof on nerve impulse transmission, 431
- Metabolism, medulla, 135
- , oxygen tension, 135
- , spinal cord, 135
- MINATOYA, H, See GELLHORN, E, 161
- Monkey, absence of areas 4 and 6, 181
- , area 13, 349
- , basal ganglia lesions, 405
- , cerebral cortex stimulation, 181
- , decorticate, EEG, 233
- , electroencephalogram, 405
- , hemisection, 155
- , hyperactivity, 349
- , hyperphagia, 349
- , hypothalamus lesions, 405
- , long association fibers, 129
- , motor response, 181

# INDEX

- Accommodation, sensory fibers, 333
- Acetylcholine, formation, 397
- level, anoxia, 329
- , —, hypoglycemia, 329
- removal by cholinesterase, 431
- Action potential, phosphocreatine, 383
- ADES, H W A secondary acoustic area in the cerebral cortex of the cat, 59
- , See MARSHALL, W H, 1
- Alternation, delayed, effect of cortical lesions, 173
- , —, in rat, 173
- Anoxia, acetylcholine level, 329
- Area, acoustic, in cerebral cortex, 59
- 4, absence in monkey, 181
- 6, absence in monkey, 181
- 13, hyperactivity, 349
- , —, hyperphagia, 349
- , —, monkey, 349
- , tactile sensory, 287
- Auditory-nerve fibers, acoustic stimulation, 39
- Authorhythmic mechanism, sensory fibers, 337
- BAILEY, P, BONIN, G v, GAROL, H W, and McCULLOCH, W S Functional organization of temporal lobe of monkey (*Macaca mulatta*) and chimpanzee (*Pan satyrus*), 121
- , —, —, —, Long association fibers in cerebral hemispheres of monkey and chimpanzee, 129
- BARD, P, See WOOLSEY, C N, 287
- BEATON, L E, and LEININGER, C R Spinal distribution of thermoregulatory pathways in monkey, 37
- , —, and MCKINLEY, W S Thermoregulatory pathways in the cat brain, 29
- , see CANNON, B W, 425
- BEECHER, H K, See CRAIG, F N, 135
- BISHOP, G H Responses to electrical stimulation of single sensory units of skin, 361
- BONIN, G v, See BAILEY, P, 121, 129
- Brain stem, cat, 29
- BRITTON, S W, and KLINE, R F The pseudoaffective state and decerebrate rigidity in the sloth, 65
- BULLOCK, T H, BURR, H S, and NIMS, L F Electrical polarization of pacemaker neurons, 85
- BURR, H S, See BULLOCK, T H, 85
- Calcium, intravenous, electrogram of cat, 23
- CANNON, B W, BEATON, L E, and RANSON, S W, Jr Nature of paresis following lateral cortico-spinal section in monkeys, 425
- CASE, T J, See WALKER, A E, 213
- Cat, acoustic area, 59
- , anesthetized, cortical response, 1
- , brain stem, 29
- , electrogram of, intravenous potassium, calcium, magnesium, 23
- , hind limb reflexes, 298
- , neuron patterns, 298
- , salivatory center, 195
- , spinal, response to stretch, 317
- , thermoregulatory pathways, 29
- Cerebral hemispheres, chimpanzee, 129
- , —, monkey, 129
- Chimpanzee, cerebral cortex, 287
- , long association fibers, 129
- , tactile sensory area, 287
- , temporal lobe, 121
- Choline acetylase, 397
- Cholinesterase, removal of acetylcholine by, 431
- COATES, C W, See NACHMANSOHN, D, 383
- Cortex, cerebral, acetylcholine level, 329
- , —, acoustic area, 59
- , —, chimpanzee, 287
- , —, hypothalamic lesions, 81
- , —, metabolism and oxygen tension, 135
- , —, motor response, 181
- , —, oxygen tension, 135
- , —, rat, 329
- , —, tactile sensory area, 287
- Cortico-spinal section, paresis, 425
- COX, R T, See NACHMANSOHN, D, 383
- CRAIG, F N and BEECHER, H K The effect of oxygen tension on the metabolism of cerebral cortex, medulla and spinal cord, 135
- DAVIS, H, See GALAMBOS, R, 39
- , See WEISS, P, 269
- EATON, B C, See RICHTER, C P, 417
- Electric organ, action potential, 383
- Electroencephalogram, decorticate monkey, 233
- , monkey, 405
- , basal ganglia lesion, 405
- , hypothalamus lesion, 405
- , thalamus lesion, 405
- Electrogram, intravenous potassium, calcium, magnesium, 23
- Electrophorus electricus*, phosphocreatine, 383
- Endplate region, excitability, 99
- Enzymes, localization, 203
- , respiratory, 203

- STEINBACH, H B , See NACHMANSOHN, D , 203
- STEWART, W B , See MCCOUCH, G P , 155
- Stimulation, acoustic, 39
- , afferent, cortical response, 1
- , ———, peripheral source, 111
- , ———, reflex action, 111
- , gross photic, cortical response, 1
- , photic, 213
- , sympathetic, sweat gland, 191
- Stretch, reflex response, 317
- Sweat gland response, 191
- Symbolic processes, rat, 173
- Synapses, visceral afferent, 263
- TALBOT, S A , See MARSHALL, W H , 1
- Temperature, reflexes, spinal cord, 225
- Temporal fusion mechanism, 213
- Thalamus, electrical activity, 243
- , lesion, electroencephalogram, 405
- Thermoregulatory pathways, cat, 29
- ———, monkey, 37
- Visual structures, electrical activity, 213
- ———, photic stimulation, 213
- Volleys, antidromic, spinal motor nucleus, 143
- , orthodromic, spinal motor nucleus, 143
- WALKER, A E , WOOLF, J I , HALSTEAD, W C , and CASE, T J Mechanism of temporal fusion Effect of photic stimulation on electrical activity of visual structures, 213
- WANG, S C Localization of the salivatory center in the medulla of the cat, 195
- WEBSTER, J E , and GURDJIAN, E S Acute physiological effects of gunshot and other penetrating wounds of the brain, 255
- WEISS, P , and DAVIS, H Pressure block in nerves provided with arterial sleeves, 269
- WELSH, J H Acetylcholine level of rat cerebral cortex under conditions of anoxia and hypoglycemia, 329
- WHELAN, F , See RICHTER, C P , 191
- WINKLER, A W , See RUBIN, M A , 23
- WOOD, W M , See MORGAN, C T , 173
- WOODRUFF, B G , See RICHTER, C P , 417
- WOOLF, J I , See WALKER, A E , 213
- WOOLSEY, C N , MARSHALL, W H , and BARD, P Note on organization of the tactile sensory area of cerebral cortex of chimpanzee, 287
- Wounds, gunshot, physiological effects, 255
- , penetrating, physiological effects, 255

- , paresis, 425
- , temporal lobe, 121
- , thermoregulatory pathways, 37
- , transection of spinal cord, 155
- MORGAN, C T, and WOOD, W M Cortical localization of symbolic processes in the rat II Effect of cortical lesions upon delayed alternation in the rat, 173
- MORISON, R S, FINLEY, K H, and LOTHROP, G N Spontaneous electrical activity of thalamus and other fore-brain structures, 243
- Motoneurons, phrenic, repetitive activity, 439
- Motor response, cerebral cortex, 181
  - , —, monkey, 181
- Muscle, denervated, excitability, 99
  - , normal, excitability, 99
  - , skeletal, effect of fixation, 17
- NACHMANSOHN, D, COX, R T, COATES, C W, and MACHADO, A L Action potential and enzyme activity in the electric organ of *Electrophorus electricus* II Phosphocreatin as energy source of the action potential, 383
  - , and MACHADO, A L The formation of acetylcholine A new enzyme "choline acetylase," 397
  - , STEINBACH, H B, MACHADO, A L, and SPIEGELMAN, S Localization of enzymes in nerves II Respiratory enzymes, 203
- Nerve impulse transmission, acetylcholine, 431
  - , localization of enzymes, 203
  - , pressure block in, 269
- Neuron, pacemaker, and electrical polarization, 85
  - pattern, 298
- NIMS, L F, See BULLOCK, T H, 85
- Nucleus, spinal motor, volleys in, 143
- OBRADOR, S Effect of hypothalamic lesions on electrical activity of cerebral cortex, 81
- OZORIO DE ALMEIDA, M Investigation of epileptiform attacks produced by sudden cooling of frog spinal cord, 73
  - , Temperature effects on reflexes of isolated spinal cord Heat paralysis and cold paralysis, 225
- Pan satyrus*, See Chimpanzee
- Paralysis, cold 225
  - , heat, 225
- Paresis, lateral corticospinal section, 425
- PARTRIDGE, R C, See SOLANDT, D Y, 17
- Phosphocreatine action potential, 383
  - , *Electrophorus electricus*, 383
- PRITS, R F The basis for repetitive activity in phrenic motoneurons, 439
- Polarization, electrical of neurons, 85
- Potassium, intravenous, electrogram of cat, 23
- Pressure block in nerve, 269
- Pseudoeffective state, decerebrate rigidity, 65
- RANSON, Stephen Walter, *obituary*, 223
- RANSON, S W, Jr, See CANNON, B W, 425
- Rat, cortical localization, 173
  - , delayed alternation, 173
  - , symbolic processes, 173
- Reflex action, afferent stimulation, 111
  - , conditioned, insulin hypoglycemia, 161
  - , hind limb, cat, 298
  - response, conduction, 317
    - , spinal cat, 317
    - , stretch, 317
    - , synaptic transmission, 317
- RICHTER, C P, and WHELAN, F Sweat gland responses to sympathetic stimulation studied by galvanic skin reflex method, 191
  - , WOODRUFF, B G, and EATON, B C Hand and foot patterns of low electrical skin resistance Their anatomical and neurological significance, 417
- Rigidity, decerebrate, sloth, 65
  - , pseudoeffective state, 65
- RUBIN, M A, HOFF, H E, WINKLER, A W, and SMITH, P K Intravenous potassium, calcium and magnesium and the electrogram of the cat, 23
- RUCH, T C, and SHENKIN, H A The relation of area 13 on orbital surface of frontal lobes to hyperactivity and hyperphagia in monkeys, 349
- Salvatory center, cat, 195
- SHEEHAN, D, See FREUND, S, 263
- SHENKIN, H A, See RUCH, T C, 349
- Skin, electrical stimulation, 361
  - reflex, stimulation galvanically, 191
  - resistance, hand and foot patterns, 417
  - , sensory units, 361
- SKOGLUND, C R, See GRANIT, R, 337
- SMITH, P K, See RUBIN, M A, 23
- SOLANDT, D Y, PARTRIDGE, R C, and HUNTER, J The effect of skeletal fixation on skeletal muscle, 17
- SPIEGELMAN, S, See NACHMANSOHN, D, 203
- Spinal cord, epileptiform attacks, 73
  - , —, isolated, reflexes, 225
  - , —, oxygen tension, 135
  - , —, sudden cooling, frog, 73
  - , —, transection monkey, 155

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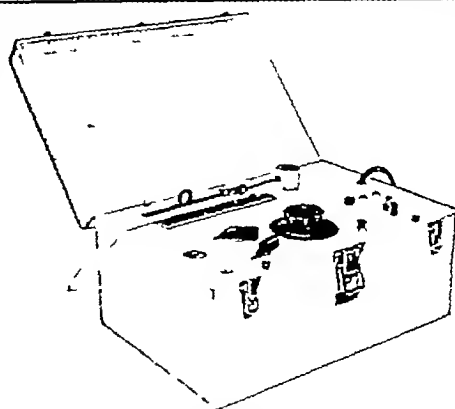
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